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THE OCCURRENCE OF VIRULENT TUBERCLE BACILLI IN PRESUMABLY NON-TUBERCULOUS LUNG TISSUE *

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In 1927 Opie and Aronson [1] reported the results of a comprehensive study to determine whether or not latent focal lesions of pulmonary tuberculosis contain virulent tubercle bacilli. A total of 304 lesions obtained from the lungs of 169 individuals dying of causes other than tuberculosis in two Philadelphia hospitals was examined. Data pertaining to 208 lesions of the primary complex from the lungs and tracheobronchial lymph nodes, which were used to inoculate guinea pigs, revealed that tubercle bacilli were present in approximately 26 per cent. Since the material examined by Opie and Aronson consisted of focal lesions that were caseous, caseocalcareous, calcified or encapsulated, the authors questioned whether the tubercle bacilli, in the instances in which the results of the inoculation of guinea pigs were positive, were present within the structure of the focal lesions or in the peripheral tissue in which the lesions were embedded. They accordingly investigated this phase of the problem, injecting guinea pigs with (1) lung tissue from the apexes, which were grossly without evidence of tuberculosis or fibrous scars; (2) lung tissue presumably free from tuberculous lesions, from the base of the lungs; and (3) apparently non-tuberculous lymph nodes from the hilum or the tracheobronchial tree. It was found that tubercle bacilli were present in tissues removed from 15, or 45.5 per cent,

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of the 33 individuals in whom the character of latent tuberculosis consisted of fibrocaseous lesions of the apexes, fibrous scars of the apexes and caseous encapsulated or calcified nodules of the lungs and contiguous lymph nodes.

In a previous communication [2] we reported the results of a study in which attempts were made by cultural procedures and by the inoculation of guinea pigs to demonstrate tubercle bacilli in chronic tuberculous lesions of the lungs and contiguous lymph nodes of individuals dying of causes other than tuberculosis. Material from a total of 68 cases was utilized and tubercle bacilli were demonstrated in only 1 instance. In the study just referred to an attempt was made in so far as was practical to use for subsequent study only those tissues that constituted the lesions of tuberculosis and to discard as much as possible of the surrounding tissues which, while apparently non-tuberculous, were found in Opie and Aronson's [1] series to contain virulent tubercle bacilli in a high percentage of cases. The occurrence in this study of only 1 positive result in material from 68 autopsies suggested the desirability of an additional study in which would be investigated the possibility of virulent tubercle bacilli occurring, as Opie and Aronson had found, in the presumably non-tuberculous tissues of the lung and contiguous lymph nodes.

Material and Methods

Only material from unembalmed bodies was selected. The specimens for subsequent study were obtained at the time of autopsy and a separate set of sterile scissors and forceps was used for the removal of each specimen. Each specimen was placed in a sterile container for transmission to the laboratory.

With one exception only bodies of persons dying of causes other than tuberculosis were included in the study. With the one exception noted, the individuals from whom the material was obtained showed no clinical signs of tuberculosis or anatomical evidence of active tuberculosis at the time of autopsy.

The material was obtained at autopsy from 51 individuals. In all but 12, lesions of so-called latent or chronic tuberculosis were noted at the time of the postmortem examination. In most instances the signs of tuberculosis consisted grossly of non-active or presumably healed lesions usually referred to as the Ghon or

primary complex. A summary of lesions that were designated as tuberculous in character is as follows: healed Ghon complex of the lungs with no lesions of tuberculosis elsewhere, 21 cases; healed Ghon complex with healed lesions in the liver alone or in the liver and spleen in combination, 5 cases; Ghon tubercle of lung only, 1 case; healed lesions of the hilar lymph nodes only, 8 cases; healed lesions of the hilar lymph nodes and liver, 2 cases; and healed tuberculosis of the apical lobe, 1 case. (In 1 case the patient had tuberculous apical scars of the lungs and died of a tuberculous enteritis and peritonitis.) In 25 of the cases the lesions of tuberculosis were associated with so-called "apical scars."

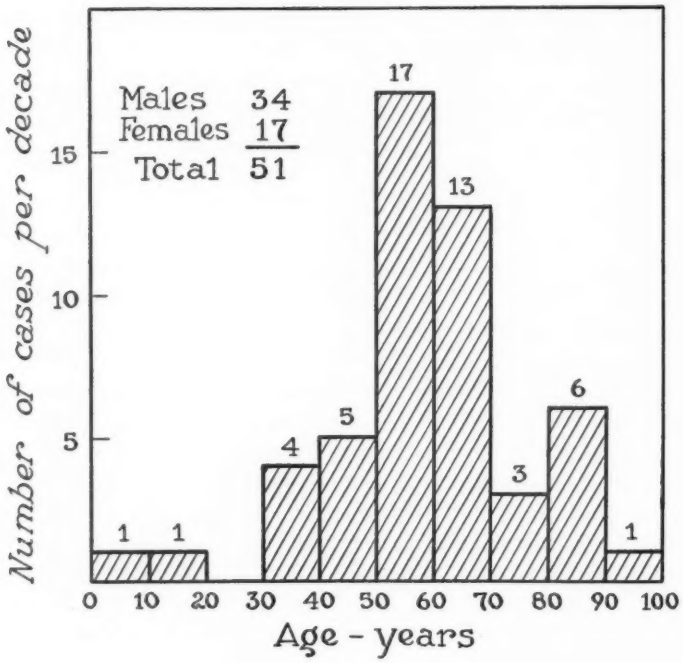
The ages of the respective individuals comprising the 51 cases ranged from 2½ to 93 years with the largest number between 50 and 69 years inclusive (Text-Fig. 1). All were white. Thirty-four were males and 17 were females. The environment of 13 was rural and of 38 urban.

In securing material to be examined for the presence of tubercle bacilli only tissue that appeared grossly to be non-tuberculous was selected. If focal lesions of presumably healed tuberculosis were present, care was taken to select the specimen at some distance from the focal lesions rather than immediately adjacent to them. Although the procedure followed varied slightly in a few instances, the material obtained from each body consisted of portions of both upper and both lower lobes of the lungs and the hilar or tracheobronchial lymph nodes.* A composite emulsion was prepared from both of the upper lobes and the same procedure was followed for both of the lower lobes. Hilar or tracheobronchial lymph nodes were prepared as one emulsion. In most instances, therefore, there was available from each case 3 emulsions for inoculation into guinea pigs.† The entire liquid portions of the respective emulsions were divided between 2 guinea pigs, the injections being made subcutaneously. The guinea pigs were kept un-

* An idea of the amount of lung tissue secured for study may be obtained from the following. In 25 of the cases the average weight of the tissue from both upper lobes was 11.2 gm. and the average weight of the tissue from both lower lobes was 14.5 gm.

† Cultures were also attempted from the emulsions prepared from the first 25 cases, but since the results were all negative it was thought advisable to utilize all of the subsequent material for the injection of guinea pigs.

der observation for 8 weeks, after which time they were killed and examined for evidence of infection with tubercle bacilli. At the time of autopsy the guinea pigs were examined carefully and any abnormality that appeared to resemble tuberculosis even remotely was submitted to subsequent study, which included his-



TEXT-FIG. 1. Age distribution by decades of the 51 individuals from whom material was obtained for study.

tological examination, cultural attempts to demonstrate tubercle bacilli, and in some instances the inoculation of additional guinea pigs with an emulsion of the tissue in question.

A summary of the results of the animal inoculation tests shows that from the 51 cases there was prepared for the inoculation of guinea pigs a total of 150 emulsions. These consisted of 51 composite emulsions prepared from portions of both upper lobes, 51 composite emulsions prepared from portions of both lower

lobes, and 48 composite emulsions of hilar and tracheobronchial lymph nodes. A total of 300 guinea pigs was inoculated and 265 of these lived 3 weeks or longer after being inoculated. In fact, death among the inoculated guinea pigs from intercurrent infections seldom occurred after the first 3 weeks. Although 35 guinea pigs were recorded as "failures," since they died within 3 weeks after being inoculated, the majority of the animals were living at the end of 8 weeks, when they were killed. In only 6 instances did both guinea pigs that were inoculated with the same emulsion die during the first 21 days. In no instance did all of the guinea pigs in a group inoculated with material from the same case die before the period usually necessary for the recognition of the lesions of experimental tuberculosis.

Results

From the results of the autopsy findings in the guinea pigs, tubercle bacilli were demonstrated in only 3 of the 51 cases (Table I). The salient features concerning the clinical history and the pathological findings in these cases follow.

Case 1: This patient, a white woman of 78 years, had been confined in a psychopathic hospital for 25 years because of a manic depressive psychosis. In 1936 her neck had become swollen and inflamed and persistent draining sinuses had developed. In July, 1937, she suffered a fracture of the neck of the right femur. Following this she failed gradually. Two days before death she had numerous tarry stools.

At autopsy tuberculous enteritis and peritonitis with a severe gastro-intestinal hemorrhage were found. There was a tuberculoma between the stomach and the pancreas, and tuberculous nodules were found in the liver and in the lymph nodes of the right supraclavicular region. The cervical lymph nodes were not examined.

The only gross evidence of tuberculosis of the lungs was bilateral apical scarring graded 3 on a basis of 1 to 4. The scar at the right apex was calcified.

Histological Examination: The section of the decalcified right apical scar revealed a small mass of hyalinized connective tissue partially surrounded by a small group of lymphocytes. There were many dilated bronchioles embedded in a basophilic connective tissue. Groups of lymphocytes were numerous around the bronchioles but nowhere was there evidence of active tuberculosis. The

section of the left apical scar had a similar appearance. None of the sections from the lung or hilar nodes taken from regions contiguous to those from which specimens were taken for inoculation revealed any evidence of tuberculosis either active or healed.

TABLE I

Summary of the 3 Cases from which Virulent Tubercle Bacilli Were Demonstrated

Case	Age	Sex	Tuberculosis at autopsy	Inoculum *	Results
1	*yrs.* 78	Female	Bilateral apical scars. Miliary tubercles in liver and spleen. Tuberculous enteritis and peritonitis	Upper lobes of both lungs exclusive of apical scars	Both guinea pigs tuberculous
				Lower lobes of both lungs	Both guinea pigs negative
				Hilar lymph nodes	Both guinea pigs negative
2	65	Female	Healed primary complex of lungs. Bilateral apical scars	Upper and lower lobes of right lung	Both guinea pigs negative
				Upper and lower lobes of left lung	One guinea pig negative. Second guinea pig tuberculous and culture obtained from spleen
				Hilar lymph node	Both guinea pigs negative
3	85	Male	Healed primary complex of lungs. Bilateral apical scars	Upper lobes of both lungs	One guinea pig negative. Second guinea pig died after 2 days
				Lower lobes of both lungs	Both guinea pigs negative
				Hilar lymph nodes	One guinea pig negative. Second guinea pig tuberculosis limited to spleen

* Each inoculum prepared from the lungs represented approximately 10 to 14 gm. of tissue.

Sections of the organs grossly involved by tuberculosis revealed evidence of an active tuberculous process attended with much caseation. In addition to the organs involved grossly, histological examination of the spleen revealed occasional tubercles.

Animal Inoculations: Material for the inoculation of guinea pigs consisted of tissue from the apparently normal part of the upper lobes of both lungs, the same from both lower lobes, and lymphoid tissue from the region of the hilum. Six guinea pigs were injected and in only 2 were lesions of tuberculosis found when they were killed 8 weeks later. Both of the animals in which lesions of tuberculosis were found had received portions of the composite emulsion prepared from the upper lobes of the lungs.

The tuberculosis present in 1 of the guinea pigs was minimal in amount, while in the other animal there was extensive involvement of the spleen and a few tuberculous foci in the lungs. From the spleen of this animal a culture with the physical characteristics of the human form of the tubercle bacillus was obtained.

CASE 2: This patient, a white woman of 65 years, had previously been a resident of a city of 30,000 population. There was no history of tuberculosis in the immediate family. She had had convulsions about once a month since the age of 34 years. She was poorly nourished and disoriented. Nine days before her death she was committed to a psychopathic hospital. She failed gradually and died after repeated convulsions.

At autopsy it was found that she had an ancient cerebral infarction in the right occipital region with a dilatation of the posterior horn of the left lateral ventricle. She was emaciated grade 3 +. The only anatomical evidence of tuberculosis was the presence of bilateral apical scars graded 1 + and an apparently healed Ghon complex. The Ghon tubercle was located in the lower lobe of the right lung and the lesion in the hilar node was small and calcified. These lesions were not found grossly until after they had been located by roentgenographic studies of the lungs removed at autopsy.

Histological Examination: Sections taken from regions contiguous to the specimens removed for inoculation experiments showed no evidence of tuberculosis, either active or healed. No sections were taken of the Ghon tubercle or the corresponding lesion in the hilar node.

Animal Inoculations: This was one of the few instances in which material from the upper and lower lobes of the same lung was used to inject guinea pigs. The usual procedure was to prepare a composite emulsion from tissue from both upper lobes and a second emulsion using tissue from both lower lobes. Six guinea

pigs were injected as follows: 2 with the combined material from the upper and lower lobes of the right lung, 2 with the combined material from the upper and lower lobes of the left lung, and 2 with lymphoid tissue from the region of the hilum. All of the guinea pigs lived for the required observation period of 8 weeks. At autopsy all of the guinea pigs were free of demonstrable lesions of tuberculosis except 1. This animal had received an inoculum prepared from the upper and lower lobes of the left lung. The tuberculous lesions in this guinea pig consisted of a large number of tuberculous nodules in the spleen, a few small foci in the liver, and a moderate number of tuberculous foci in the lungs. The tuberculous character of the lesions was confirmed by a microscopic examination of the respective organs and by the isolation from the spleen of virulent acid-fast bacilli.

The character of the culture obtained was dissimilar to that of the usual strains of tubercle bacilli of the human type and was subjected to further study. Although material from the tuberculous spleen of the guinea pig was used to inoculate both glycerinated and non-glycerinated mediums, growth occurred only on the mediums that contained no glycerin.* Of the four tubes of non-glycerinated medium inoculated with material from the spleen of the guinea pig, growth was noted 73 days later in 3. The growth consisted of a few small discrete colonies in two tubes and innumerable small colonies on another. The colonies were nonchromogenic and even inclined to be colorless and had a luster. Miscibility in a saline solution was poor. The non-glycerophilic character of the culture was maintained in subsequent transfers.

The failure of the culture to grow in the presence of glycerin, which substance appears to enhance the growth-producing propensities of most culture mediums for the human form of the tubercle bacillus, provided sufficient reason to determine the pathogenicity of the culture for rabbits. Consequently 2 rabbits and 2 additional guinea pigs were each inoculated with 0.01 mg. of the culture obtained from the spleen of the guinea pigs previously mentioned.†

The results of these tests of pathogenicity indicated quite defi-

* The medium used was the egg yolk-agar combination described originally by Capaldi [3] and more recently by Herrold.[4]

† The guinea pigs were injected subcutaneously, the rabbits intravenously.

nitely that the infective organism was a tubercle bacillus of the bovine type.* One of the rabbits died after 20 days and the other after 22 days. The lesions in the 2 animals were essentially alike. In both animals the spleen was swollen — in 1 tremendously so — and the lungs were literally filled with innumerable miliary foci indicative of aggravated and progressive tuberculosis.

CASE 3: This patient, a white man of 85 years, had lived in a city of 400,000 population and had been confined to a psychopathic hospital 3 months previous to his death because of a senile psychosis. There was no history of tuberculosis in his family. He remained bedfast and developed bronchopneumonia, from which he died. At autopsy he was found to have generalized and cerebral arteriosclerosis with multiple infarcts of the brain. There was bilateral bronchopneumonia and grade 3 + coronary sclerosis with chronic infarction of the interventricular septum of the heart.

The only gross evidence of tuberculosis of the lung was bilateral apical scarring, grade 1 +, a very small calcified lesion in a hilar node of the right lung and a hyalinized tubercle measuring 2 mm. in diameter in the liver.

Histological Examination: Sections of the apical scars revealed no evidence of tuberculosis. Sections taken from regions in the lungs and hilar nodes contiguous to those from which tissue was removed for the inoculation experiments revealed no evidence of tuberculosis. No sections were taken of the lesions of the hilar node or the liver.

Animal Inoculations: Two guinea pigs were inoculated with material from the combined specimens of tissue from the upper lobe of each lung, 2 with the material prepared from the combined specimens of tissue from the lower lobe of each lung, and 2 with an emulsion prepared from the lymphoid tissue from the region of the hilum. One of the guinea pigs inoculated with material from the upper lobes died 2 days later. The other 5 guinea pigs were living at the end of 8 weeks when they were killed for autopsy. In only 1 of them was evidence of tuberculosis found. This animal was 1 of the 2 that had been inoculated with material prepared from the lymphoid tissue from the hilum. Grossly the spleen showed four or five nodular lesions, apparently composed of conglomerate tubercles. The liver and lungs were not involved,

* In order to verify this conclusion the organism was "typed" a second time using guinea pigs, rabbits and chickens. The results indicated that the organism was not pathogenic for chickens but was markedly so for guinea pigs and rabbits.

either grossly or microscopically. Microscopically the character of the changes in the spleen of the guinea pig was that of a mild rather than a severe tuberculous infection. There occurred diffuse nodules of epithelioid cells and histiocytes with a minimal amount of necrosis. A considerable portion of the spleen was apparently normal. In appropriately stained sections a few typical acid-fast bacillary forms were noted.

An attempt was made to secure cultures of tubercle bacilli from the spleen but it was unsuccessful. A portion of the splenic emulsion was used to inoculate 2 additional guinea pigs, 1 of which died 38 days and the other died 54 days after inoculation. Both animals showed a limited tuberculosis of the spleen and in 1 the liver was also slightly involved. Cultures were attempted from the spleen of the animal that died after 38 days and many colonies appeared on both glycerinated and non-glycerinated mediums. The characteristics of the cultures were those of the human tubercle bacillus.

Comment

In addition to the report of Opie and Aronson,[1] previously mentioned, there are relatively few reports of investigations dealing with the latent phase of tuberculous infections. This is especially true as regards the question of latency of pulmonary infection, in which search for tubercle bacilli has been made of the presumably non-tuberculous parenchyma of the lung.[5]

In 1890 Loomis [6] examined by the intrapleural inoculation of rabbits the apparently normal bronchial lymph nodes of 30 adults and obtained positive results in 8. Pizzini [7] in 1892 demonstrated by guinea pig inoculations the presence of tubercle bacilli in 12 of 30 adults in whom visible lesions of tuberculosis were not evident. Ten of the 12 positive results were obtained from bronchial lymph nodes and 2 from cervical lymph nodes. In these latter 2 the bronchial nodes also gave positive results.

In a report by Spengler [8] in 1893 an account was given of the finding by histological methods of tubercle bacilli in the bronchial lymph nodes of 6 children who had died of acute febrile diseases. Other evidence of tuberculous infection was not observed. Straus [9] in 1894 studied material from the nasal passages of 29 individuals whose duties required their presence for different periods of time

in a room occupied by tuberculous patients. Virulent tubercle bacilli were demonstrated 9 times.

Kälble [10] in 1899 reported the demonstration of tubercle bacilli by guinea pig inoculation tests from the presumably normal bronchial lymph nodes in 2 cases of 23 investigated. One of the positive cases was an adult, 41 years of age, and the other a child 5½ years of age. The material utilized by Kälble was examined grossly and microscopically for lesions of tuberculosis, but no evidence of infection was noted.

Wang [11] quoted a study by Griffith [12] which included material from a series of 61 children who showed at autopsy no morbid signs of a tuberculous infection. The bronchial and mesenteric lymph nodes from each individual, and in one instance the cervical lymph nodes also, were used to inoculate guinea pigs. Material from 5 of the cases produced tuberculosis in the test animals and the infective agent in 3 of these was found to be of the bovine type.

Griffith [12] investigated the infectivity of bronchial and mesenteric lymph nodes of children who failed to show morbid signs of tuberculosis at autopsy. Material from 34 of the cases was studied by inoculation into guinea pigs and the bronchial lymph nodes of 2 of the children were found to contain virulent tubercle bacilli of the human type.

Recently (1938) Saenz and Canetti [13] reported having demonstrated tubercle bacilli in 1 of 14 specimens from apparently normal lungs. They concluded that tubercle bacilli were present but rarely in the tissues of presumably normal lungs.

This résumé of the literature, while not complete, indicates that occasionally tissues such as the parenchyma of the lung or the contiguous lymph nodes may contain virulent tubercle bacilli although morbid signs of the infection are not detectable. However, whether the presumably non-tuberculous parenchyma of the lung does or does not contain tubercle bacilli cannot be established with finality. The technical difficulties presented preclude the examination by any available method of other than a very small portion of the total substance of the lung. That positive results are obtained occasionally from relatively small portions of the organ prompts the suggestion that tubercle bacilli might be demonstrated more frequently if it were possible to use the entire paren-

chyma for test purposes. However, if the lung tissues of clinically and anatomically non-tuberculous individuals do contain tubercle bacilli in any considerable percentage of cases, it is difficult to account for the apparent lack of clinical or morbid signs of disease as a consequence of the presence of such bacteria. It may be, as some contend, that the natural resistance imparted as a consequence of the primary complex is operative and is successful in most instances in preventing the development of foci of reinfection.

It is not surprising that the atmosphere of an environment in which there is a relatively large number of tuberculous individuals should at times contain tubercle bacilli. It has been shown, however (Fishberg,[14] 1932), that in adults such exposure to the infective agent is rarely followed by recognizable evidence of disease. However, in those instances in which the infective bacteria are proved to be present in the tissues with no clinical or pathological signs of tuberculosis, it is conceivable that during life they might constitute a hazard to susceptible individuals.*

The fact that the 3 cases in our series which yielded positive results represented individuals who were at the time of death inmates of a psychopathic hospital is of interest. Patient 1, who had been confined to the institution for the last 25 years of life, hardly warrants consideration since the cause of death was tuberculous enteritis and peritonitis, which conditions provide sufficient explanation for the presence of tubercle bacilli in the tissues of the lung. This was probably a terminal episode since the lungs were without gross or microscopic signs of recent or active tuberculous infection.

Patient 2, however, from whose tissues tubercle bacilli of the bovine type were isolated, had been committed to the hospital only 9 days prior to death. In this instance the fact that tubercle bacilli were demonstrated in the presumably non-tuberculous parenchyma of the lungs is of less importance than the fact that the tubercle bacilli obtained were bovine rather than human in type. This would seem to be an important observation although the explanation for the possible source of the infection is obscure.

* Shrewsbury and Barson[5] raised the question of the existence of temporary or permanent "carriers" of tubercle bacilli in instances where there were no clinical signs of tuberculosis but where tubercle bacilli were demonstrated in the sputum by cultural procedures.

In this instance only 1 of the 6 guinea pigs inoculated with material from the lungs and hilar lymph nodes was tuberculous at the time of autopsy, 57 days after injection. The lesions observed at autopsy were definitely characteristic of those of "injection" tuberculosis in the guinea pig and were unlike the morbid changes that occur in tuberculosis of guinea pigs acquired spontaneously. Culturally and pathogenically the organism recovered from the tuberculous spleen of this guinea pig had the features that distinguish the bovine form of the tubercle bacillus.

It should be noted in this connection that the type of the tubercle bacillus recovered from Patient 2 would not have been identified had not cultures from the diseased spleen been attempted. This suggests the desirability of isolating and identifying the provocative agent in every instance where possible in studies of this character. Perhaps if the practice were more universally followed other strains of tubercle bacilli that are presumed to be of the human type might prove to be otherwise.

Patient 3 of our series had been a patient in the psychopathic hospital for 3 months before death. Whether the tubercle bacilli demonstrated were acquired from the environment of the institution or elsewhere is problematic.

The significance of our findings consists, we believe, not in the fact that the tissues from a small percentage of the individuals contained virulent tubercle bacilli, but rather in the fact that in most of the material examined no tubercle bacilli were found. This, we believe, is important and coincides with a rational concept of tuberculosis as the disease occurs in areas where the morbidity from tuberculosis is not high. If the reverse were generally true, one might expect a higher incidence of active infection.

A possible explanation of the differences in our findings and those of Opie and Aronson [1] may be sought in the differences in the concentration of tuberculous infections in the respective environments from which the material in the two studies was obtained. The greater part of our material came from an area where the morbidity from tuberculosis is not high, while that used by Opie and Aronson represented a portion of a population from an area where tuberculosis is, or was, more than commonly present. Furthermore, another factor that may be significant is the fact that there has been a gradual but definite decline in tuberculous

infections in the United States during the decade or more since Opie and Aronson's studies were completed.

Summary and Conclusions

Opie and Aronson having reported the demonstration of virulent tubercle bacilli in the presumably non-tuberculous lung tissue in 15 of 33 bodies examined in Philadelphia in 1927, a comparable study was made of material secured at Rochester, Minnesota. Tissues from 51 unembalmed bodies were utilized for the inoculation of guinea pigs. The age distribution was from 2½ to 93 years with the largest number of cases between 50 and 69 years inclusive. All were white. Thirty-four were males and 17 were females. The bodies selected for the study represented individuals who, with one exception, had died of causes other than tuberculosis. In 12 of the bodies no gross or microscopic evidence of tuberculosis was found, while in 38 there were lesions of latent or healed tuberculosis. In the majority of instances the signs of primary tuberculosis present were those of the primary complex of the lungs.

Material for the inoculation of guinea pigs consisted of what appeared to be non-tuberculous portions of the upper and of the lower lobes of each lung and the apparently non-tuberculous hilar lymph nodes. In all but 3 cases, 3 emulsions of tissue were prepared from each body and were used to inject 6 guinea pigs. A total of 150 emulsions was utilized to inject a total of 300 animals.

Positive results were obtained from only 3 individuals and since in 1 of these the cause of death was tuberculous enteritis and peritonitis, only 2 positive cases need be considered. In 1 case tubercle bacilli definitely identified as bovine in type were obtained from the spleen of 1 of 2 guinea pigs previously inoculated with a composite emulsion prepared from presumably normal tissues from the parenchyma of the upper and lower lobes of the left lung. In another case tubercle bacilli were demonstrated from the hilar lymph nodes.

The results of this study, made of material from an area where the morbidity from tuberculosis is not high, indicate that virulent tubercle bacilli are infrequently present in the presumably non-tuberculous tissue of the lungs of individuals dying of causes other than tuberculosis.

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FIXING AND STAINING METHODS FOR LEAD AND COPPER IN TISSUES *

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Lead and copper are of great importance clinically, especially lead, owing to their poisonous properties. Unfortunately there are no specific differential stains for these metals in tissues as there is, for example, for iron. Therefore it has not been possible to study them microscopically in direct connection with the lesions they produce. Both metals will stain gray to black if thin pieces of tissue are placed in a solution of hydrogen sulphide, but the test is not at all delicate and microscopically is useless. The object of this communication is to call attention to two simple staining methods which although only partially differential are very delicate and histologically of value.

STAINS FOR LEAD

Hematoxylin Stain: Hematoxylin and its ripened derivative hematein unite with a number of metals to form colored compounds, some of which have been found very useful as stains for nuclei and other tissue elements. As a rule hematein is required to make the stain effective. For this reason an alum hematoxylin solution must be ripened by the aid of light, heat or an oxidizing reagent. For staining lead, hematoxylin itself is essential and ripening of the staining solution must be prevented as far as possible. On this account a solution in dibasic potassium phosphate has been found most useful.

Fixation: Tissues to be examined for lead must be fixed in 95 per cent or absolute alcohol. Formalin is worthless and therefore tissues fixed in this reagent in the past are useless.

Method of Staining: Stain celloidin sections in the following solution in the paraffin oven (about 54° C.) for 2 to 3 hours, rarely for longer. Paraffin sections are loosened from the slide.

Dissolve 5 to 10 mg. (but not more) of hematoxylin in a few drops of absolute or 95 per cent alcohol and add 10 cc. of a freshly filtered 2 per cent aqueous solution of dibasic potassium phos-

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phate. After staining, wash the sections in several changes of tap water for 10 minutes to 1 hour, dehydrate in 95 per cent alcohol, clear in terpineol and mount in terpineol balsam.

Results: By this method lead is stained a light to a dark grayish blue, and the nuclei (which owe their staining properties to the presence of metals) take a deep blue color.

Other solutions of hematoxylin ripen so quickly that hematein or some intermediate product is formed and stains the lead brownish instead of blue and are therefore useless.

The best tissue for studying the effect of acute poisoning by lead is obtained by feeding rats with dog chow thoroughly soaked with a saturated (about 1 per cent) solution of lead chloride and giving them the same solution to drink. Pulverized metallic lead and lead carbonate and phosphate may also be used but act more slowly. In 8 weeks the cytoplasm of the liver cells contains numerous small, round and irregularly shaped granules which tend to fuse together to form networks and which stain clear blue by the hematoxylin method given above.

Excellent tissue for study can also be obtained by feeding *Macacus rhesus* monkeys with lead chloride but they are much more susceptible to lead poisoning than rats. A monkey given 25 cc. of a saturated solution daily on its food became partially paralyzed in its hind legs and unsteady on its feet in 8 weeks. Microscopically many liver cells were necrotic, mitotic figures were numerous and small foci of regenerated liver cells occurred here and there. In the old cells were granules of different sizes which tended to fuse together to form networks. Both the granules and the networks stained blue in the hematoxylin solution recommended above. The liver tissue showed all the microscopic changes of an early beginning cirrhosis. A smaller dose and longer time seemed all that was necessary to produce a typical cirrhosis but much time (months or years) would evidently be required.

Methylene Blue Stain: Using sections from these same experimental lesions produced by lead, it was found that the granules which stain with hematoxylin stain even more intensely by methylene blue. Staining 10 to 20 minutes in a 0.1 per cent solution in 20 per cent alcohol is sufficient and decolorization in 95 per cent alcohol takes about the same length of time. For microphotographic purposes this stain is of value.

These two staining methods were tried on sections of alcohol-fixed livers from numerous cases of alcoholic cirrhosis. The old hyalin, which is characteristic of this type of cirrhosis, was stained slightly or not at all by both methods, probably owing to the disappearance of the lead. On the other hand, many of the younger regenerated liver cells contained numerous granules and small networks which stained intensely blue and resembled closely those produced in rats and monkeys by acute poisoning with lead.

The methylene blue solution was then tried on paraffin sections of Zenker-fixed livers from rats and monkeys poisoned with lead. The granules and beginning networks stained intensely blue. The same was true of sections of livers from human cases of alcoholic cirrhosis and if the usual staining method of phloxine followed by methylene blue was employed and the methylene blue was allowed to act long enough so as to stain deeply, the old hyalin was colored various shades of red while the granules and beginning networks were stained deep blue.

The probable explanation is that the lead unites with the chrome salt present in the Zenker's fluid and the lead chromate formed is not soluble in the acetic acid and therefore persists.

Stains for Copper

Copper is fixed well by both alcohol (95 per cent or absolute) and neutral formalin, and is stained intensely blue by hematoxylin and by hematein. The simplest method is to use the same solution recommended for staining lead. The iron so commonly associated with copper stains black after alcohol fixation, but light to dark brown after formalin fixation.

Rats were poisoned with copper acetate (normal cupric) by giving them each 2 cc. of a 5 per cent aqueous solution daily on their food. The dosage was a little too large because they began to die after 4 months. Two organs, the liver and the kidney, showed marked lesions. In 2 to 3 months all the liver cells contained small granules which stained intensely blue to blue-black with hematoxylin. After that length of time the liver cells began to undergo necrosis and regeneration took place. The resulting picture after 6 months was very much like that seen in the liver from an early active case of hemochromatosis but the pigment present was copper hemofuscin, not hemosiderin, and the granules

stained blue, not black. The beginning elimination of copper and transformation of hemofuscin to hemosiderin seem to require about 7 months.

The lesion in the kidneys was equally marked. Copper hemofuscin was deposited abundantly in the cells lining the convoluted tubules and caused necrosis. The desquamated necrotic cells filled the lumens of the tubules and active regeneration occurred along the walls.

When the hematoxylin staining method was tried on sections of livers from cases of hemochromatosis it was found that the hemosiderin in the pigment stained black after alcohol, but light to dark brown after formalin fixation. In the islands of regeneration, when present, where the pigment was being laid down and was therefore freshly formed, the granules stained blue to blue-black, strongly suggesting copper.

Frequently inspissated bile was found in dilated bile capillaries. It was colored deep blue by hematoxylin, indicating copper, and clearly explains the manner in which copper gains entrance to gall stones where its presence was pointed out by Schönheimer and others.

In the kidneys from cases of hemochromatosis the lesion was much less marked than in the animals poisoned with copper, but hemosiderin was found in some of the cells of the convoluted tubules and in a few cases casts in the collecting tubules stained blue with hematoxylin, indicating copper.

When the methylene blue stain was used on sections of the experimental lesions produced in rats the copper stained blue but to only a moderate degree. The same was true of the copper in the islands of regeneration in the liver in hemochromatosis. For staining the latter lesions methylene blue has, however, one great advantage. Hemosiderin does not take the stain and therefore appears yellow to light brown. As a result the copper, in spite of its light blue color, shows up quite clearly. When the pigment granules contain both copper and iron they stain green.

Comment

For many years we have been working primarily on the subject of chronic lead poisoning and its relation to alcoholic cirrhosis, and to a less extent on chronic copper poisoning and its relation

to hemochromatosis. It seems advisable to publish at the present time the methods found useful for fixing these metals, especially lead, in the tissues and for demonstrating them by special stains. Because the staining methods are only partially differential and therefore not diagnostic, they have to be supported by a certain amount of histological and experimental work. As a result a brief summary of our results to date is presented.

Both metals, but especially copper, are very slowly acting, chronic poisons. To produce experimentally all the steps of the various changes which occur in man would require a number of years. In the meantime much valuable pathological material might be lost if not properly preserved. Alcohol is the best fixative for both metals and is absolutely necessary for demonstrating lead. Formalin is about as useful as alcohol for copper. Zenker's fluid is the best fixative for tissues to be studied histologically and preserves both metals but cannot be considered as an ideal fixative for them because the hematoxylin staining method is useless after it. The wisest procedure is to preserve tissues in all three fixatives from all important cases.

The liver would seem to act as a temporary storehouse for lead which causes little or no evident damage unless the amount absorbed exceeds a certain minimum.

Hyaline bodies were found in the nuclei of liver cells in the monkey and the hog, as in children, but were not found in the rat. They stained blue with hematoxylin like the granules in the cytoplasm of the liver cells but slightly less intensely.

The decision whether or not a case of cirrhosis is due to lead, copper or some other agent must be made by a pathologist using the best differential stains available. The patient may have had hemochromatosis and yet not have imbibed any amount of copper for months or years before death and it might have been largely or entirely eliminated during that time. Under these conditions the chemist is helpless and his results valueless unless his work is controlled by careful microscopic examination to show whether lead or copper is present or not. On the other hand, an individual may have a normal appearing liver and yet may have imbibed a large amount of lead or copper during several months preceding death, and therefore show much of one or the other metal present.

The hematoxylin staining method given here, when applied to

the central and peripheral nervous system, brings out, apparently, the same structures as the microincineration method, namely nuclei and nucleoli, Nissl bodies, and the myeloaxostroma of nerves, owing to the presence of metals in them. If lead is deposited in the nervous system in lead poisoning it would seem to occupy the same situations and simply increase the amount of mineral present.

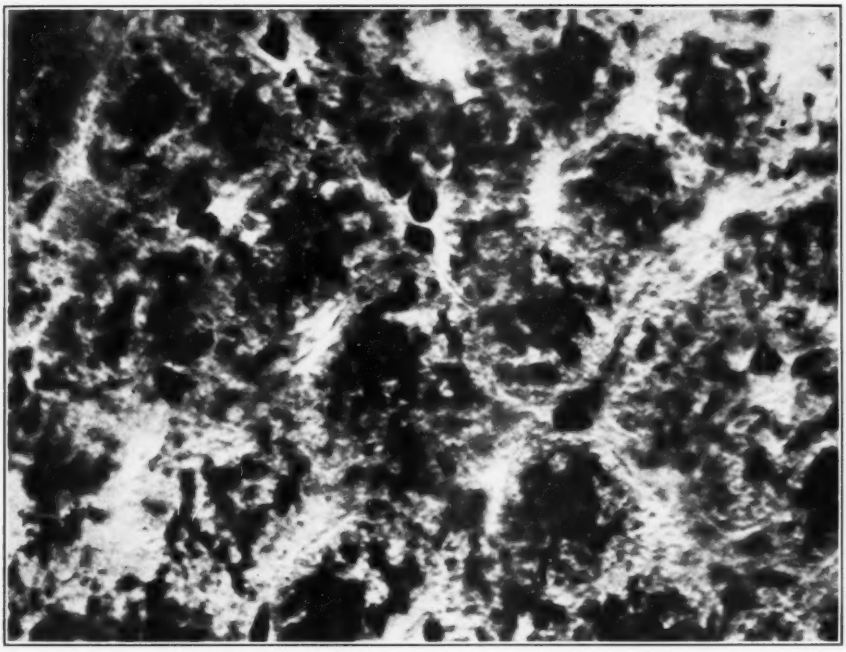
NOTE: We are indebted to Mr. Frank Dinsmore for technical assistance in making the microphotographs.

DESCRIPTION OF PLATES

PLATE 83

FIG. 1. Liver of a rat fed a maximum dose of lead chloride daily for 8 weeks. The liver cells contain granules and early hyaline networks stained blue with methylene blue. Fixation in alcohol. × 1000.

FIG. 2. Liver of a child poisoned with lead. The liver cells contain coarse granules and young hyaline networks stained deep blue with hematoxylin. Fixation in alcohol. × 1000.



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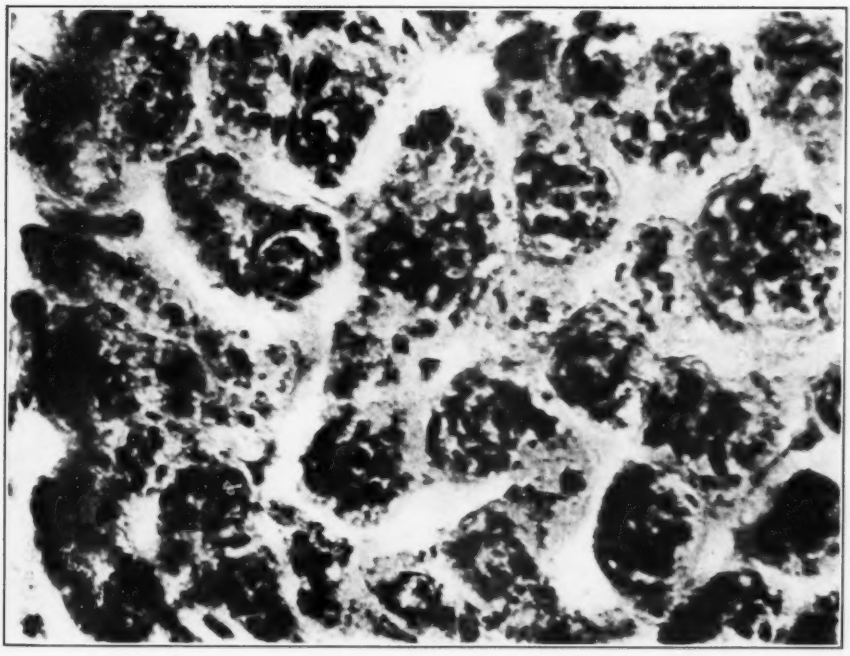

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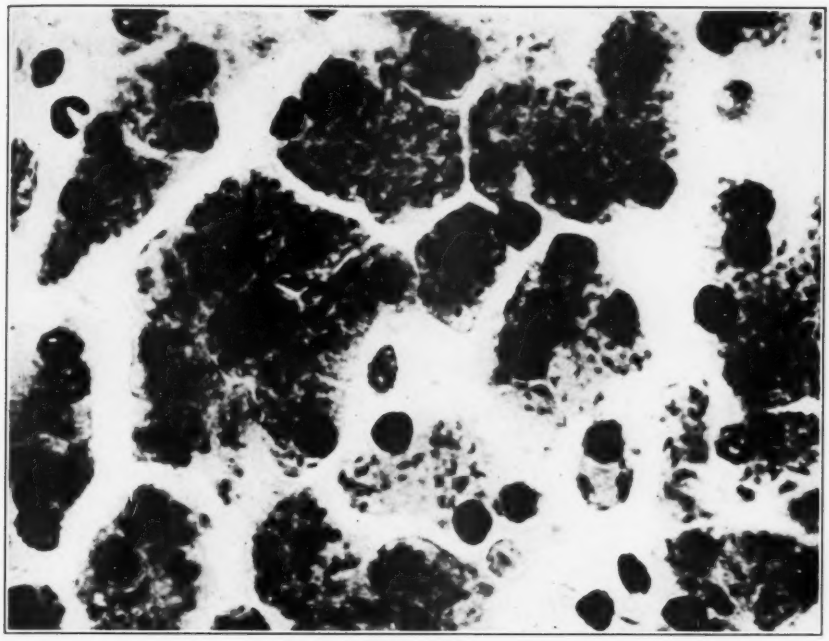
PLATE 84

FIG. 3. An island of regeneration from an active case of alcoholic cirrhosis containing much old hyalin. The liver cells are filled with granules and young hyaline networks stained intensely blue. Fixation in Zenker's fluid. Phloxine-methylene blue stain. × 1000.

FIG. 4. A normal peripheral nerve showing the myeloaxostroma as stained by the hematoxylin method recommended. × 1100.



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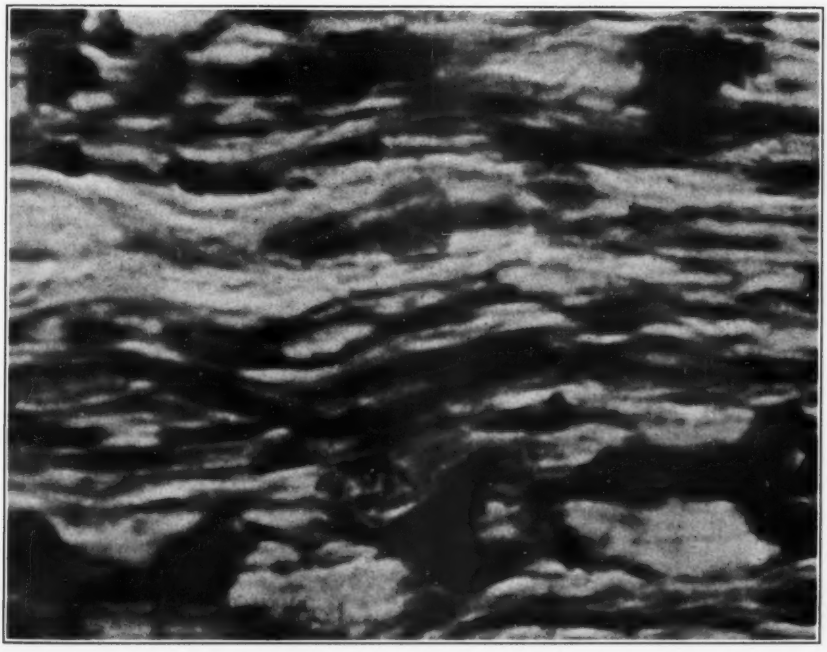

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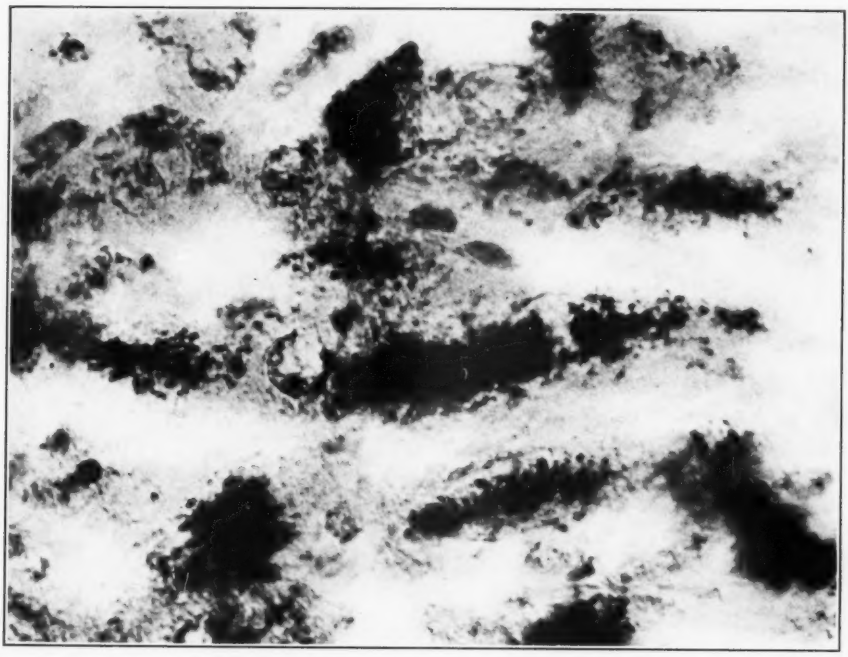
PLATE 85

FIG. 5. Liver of a rat fed cupric acetate for 6 months. The liver cells are filled with masses of granules containing copper stained a deep blue by hematoxylin. Fixation in alcohol. × 1000.

FIG. 6. Kidney of a rat fed cupric acetate for 6 months. The convoluted tubules are distended with necrotic cells filled with granules containing copper stained deep blue with hematoxylin. Fixation in alcohol. × 1000.



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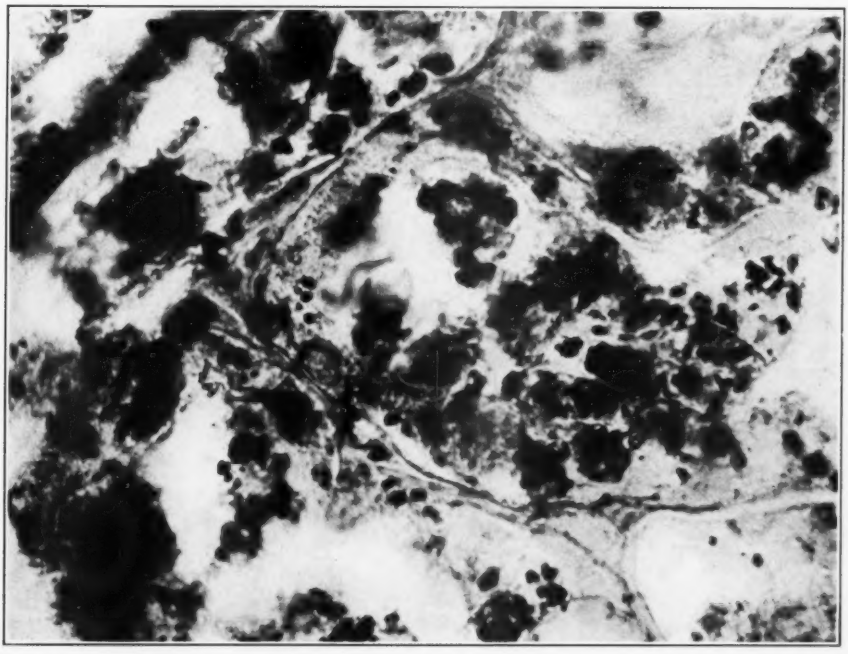

6

GIANT CELL FORMATION IN THE TONSILS IN THE PRODROMAL STAGE OF CHICKENPOX *

Report of a Case

T. H. Tomlinson, Jr., M.D.

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Alagna [1] in 1911 reported the histopathology of the nasal and pharyngeal mucosa and the lymphoid tissue in 8 children dying during the course of measles. He noted in the lymphoid follicles large cells which showed a basophilic cytoplasm and nuclei divided into two or three large masses of chromatin. He also found, without special location, nuclear masses made up of eight to fifteen nuclei lying one on top of another and with borders delineated by a thickened nuclear membrane. It was not possible to differentiate the cytoplasm in these latter structures, which he compared to the nuclei of megakaryocytes.

Next came Warthin's [2] report, published in June, 1931, and Finkeldey's [3] in August of the same year. Since then numerous reports of the occurrence of giant cells in the lymphoid tissue, especially of the tonsils and vermiform appendix, in cases of measles have appeared in the literature. However, there has been no report concerning the presence of similar cells in the tonsils and adenoids in the prodromal stage of chickenpox. The purpose of this paper is to report such a case.

Report of Case

Clinical History: L. T., a female Navajo Indian, aged 5 years, was admitted to the Southern Navajo General Hospital at Fort Defiance, Arizona, on Feb. 13, 1938, because of an infected lacerated wound of the arm. This infection cleared up and a tonsillectomy was done March 11, 1938, at the father's request. On March 14, 1938, the child developed chickenpox and remained in the hospital until March 31, 1938. There was no record of any other childhood diseases. Previous to the tonsillectomy the patient had been in a ward where several cases of chickenpox had developed.

Histopathological examination of the palatine and pharyngeal tonsils was done on March 29, 1938. There was a moderate, patchy capsular and trabecular scarring. The hyperplastic sur-

* Received for publication May 22, 1939.

face epithelium showed focal pericellular edema, patchy lymphocytic and neutrophilic infiltration, and occasional collections of pus on the surface. Embedded in this exudate on one tonsil was a mass of fungi. Occasional to numerous scattered tissue mast cells appeared in the capsule and pericapsular fibrous tissue, and in areas extended inward along the trabeculae with occasional cells lying in the adjacent pulp. In one tonsil there was a medium sized area of moderate to marked eosinophilic infiltration of the ill-defined trabeculae and adjacent pulp with occasional cells invading the surface epithelium. Here the capillaries were crowded with neutrophils. Beneath the epithelium there were scattered clumps of occasionally binucleated plasma cells, and in rare areas scattered neutrophils. There was pronounced vascular endothelial swelling. The follicles were large and hyperplastic with huge germinal centers. The reticulum cells of the follicles were swollen but not proliferated and there was much free and phagocytosed nuclear débris. The crypts did not appear to contain an unusual amount of keratin and only occasional clumps of diplococci and short chains of streptococci were seen in the surface exudate and débris.

In addition to these indications of a chronic hypertrophic tonsillitis there were numerous, scattered and grouped, large multinucleated cells. These appeared predominantly within the follicles although many were scattered in the pulp and occasional groups lay just beneath the surface and epithelium of the crypt. Occasionally in the adenoids, but rarely in the tonsils, giant cells were seen in the epithelium. These cells varied from round to oval in shape and usually had a regular outline, although in some instances it was difficult to visualize a definite limiting membrane owing to the dense packing of the nuclei. Often the innumerable overlapping nuclei completely filled the larger cells which were usually seen in the germinal centers. Frequently in the smaller cells there was some peripheral cytoplasm, but it was less abundant than is usually seen in typical foreign body giant cells. At the periphery of the follicles and in the pulp there were occasional, small to medium sized cells showing peripherally placed nuclei with small central zones of cytoplasm. The cells varied greatly in size, the largest measuring 114 by 42 μ, and the smaller ones being less than 15 μ in diameter. The nuclei measured from 4.5

to 10 μ in their long axes and varied in number from three or four to ninety or more. The majority of the cells showed no evidence of degenerative changes, and in these cells the cytoplasm was neutrophilic to slightly oxyphilic, usually finely granular but at times somewhat foamy. However, the cells in the pulp of the adenoids showed a distinctly thready, slightly basophilic cytoplasm. The nuclei, while occasionally oval, were usually round with medium sized chromatin particles radially arranged around a central nucleolus. Progressive stages in degeneration of the giant cells were seen. The nuclei became hyperchromatic and lost their reticular structure while the cytoplasm became more oxyphilic. Finally the clearly outlined cell membrane enclosed a mass of homogeneous, deeply oxyphilic cytoplasm in which were embedded clumps and strands of deeply staining, almost black, irregularly shaped and diffusely distributed nuclear débris. In some instances this débris almost filled the cell, in others it was more densely massed in the central portion, and in still others as much as half of the cytoplasm was still visible as an irregular peripheral rim or as islands distributed in the nuclear material. No phagocytosed nuclear débris, cells or microorganisms were seen within any of the giant cells.

Discussion

The giant cells seen in this case appear to be identical with those described in measles. If in this case the association of the giant cells with chickenpox rather than with measles is admitted, one must feel hesitant in accepting their presence as warranting a diagnosis of measles. In rendering the original report on this case to the clinician a probable diagnosis of measles was given, only to have the clinical history and diagnosis of chickenpox reported by him 4 weeks later. Study of additional cases of varicella is, of course, essential before any definite conclusions can be drawn. The demonstration of these giant cells in a second virus disease would seem to warrant further investigation of lymphoid tissue in other virus exanthemas.

Summary

An apparently well authenticated case of chickenpox developed in a 5 year old Indian girl 3 days after a tonsillectomy. Histo-

pathological examination of the tonsils showed numerous giant cells seemingly identical with those previously reported as occurring during the prodromal stage of measles.

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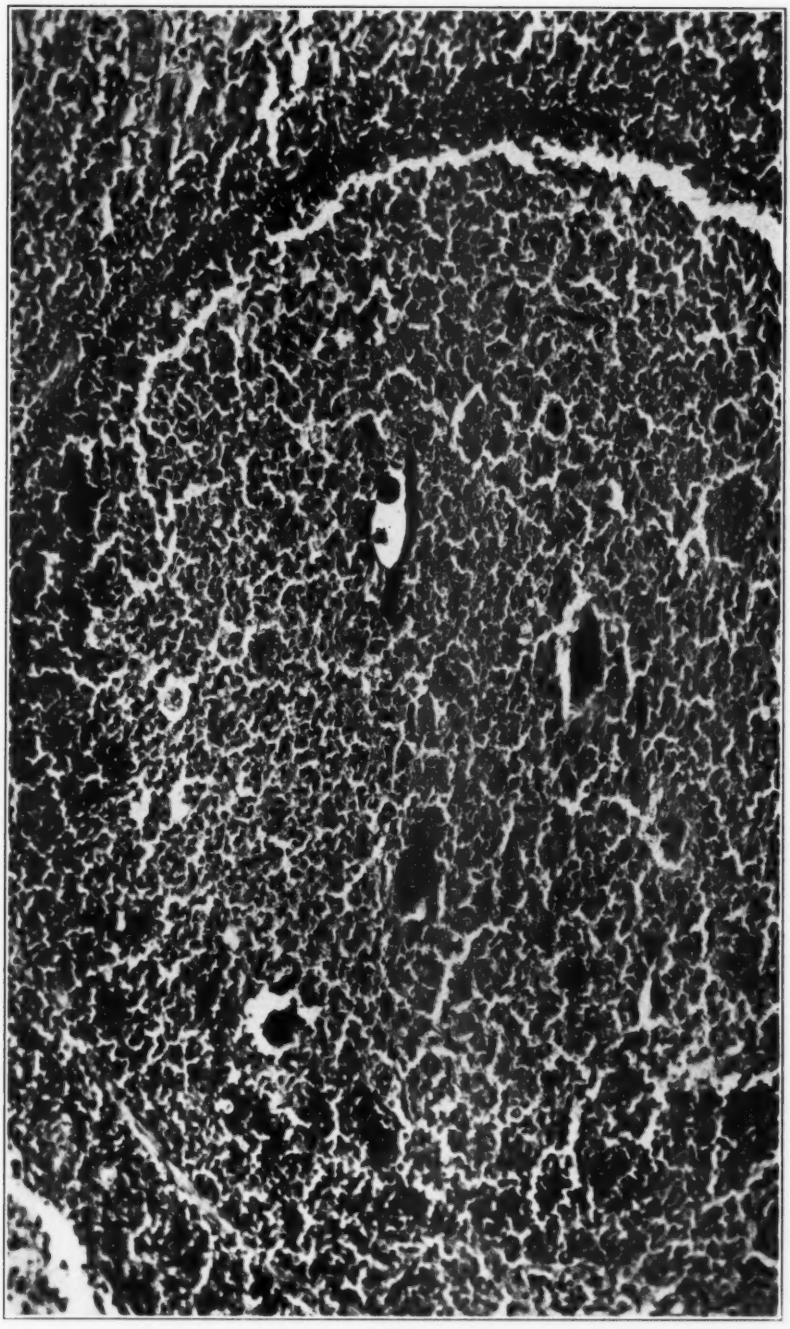
3. Finkeldey, W. Über Riesenzellbefunde in den Gaumermandeln, zugleich ein Beitrag zur Histopathologie der Mandelveränderungen im Masern-inkubationsstadium. *Virchows Arch. f. path. Anat.*, 1931, **281**, 323–329.

DESCRIPTION OF PLATES

PLATE 86

FIG. 1. (N.I.H. 1148.) A large follicle containing numerous giant cells, some of which are degenerating. Hematoxylin-Romanowsky stain. × 100.



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Tomlinson Giant Cells in Tonsils in Chickenpox

PLATE 87

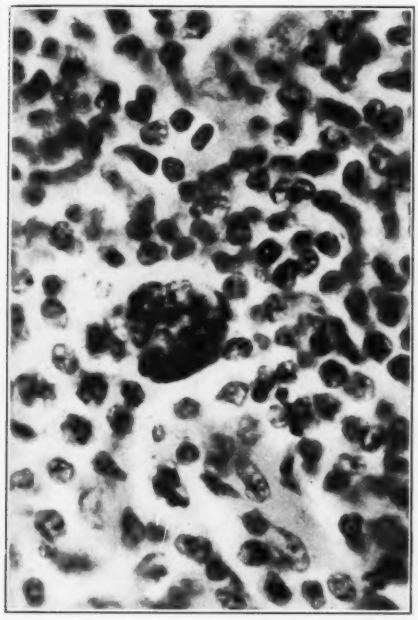
FIG. 2. (N.I.H. 1152.) A degenerating giant cell at the periphery of a follicle. Van Gieson's stain. × 700.

FIG. 3. (N.I.H. 1149.) A giant cell of the Langhans type in a follicle. Van Gieson's stain. × 700.

FIG. 4. (N.I.H. 1153). A small giant cell just beneath the epithelium of the pharyngeal tonsil. Van Gieson's stain. × 650.

FIG. 5. (N.I.H. 1150.) A giant cell in the pulp of the palatine tonsil. Van Gieson's stain. × 250.

FIG. 6. (N.I.H. 1151.) A large giant cell measuring 114 by 42 μ. Van Gieson's stain. × 250.



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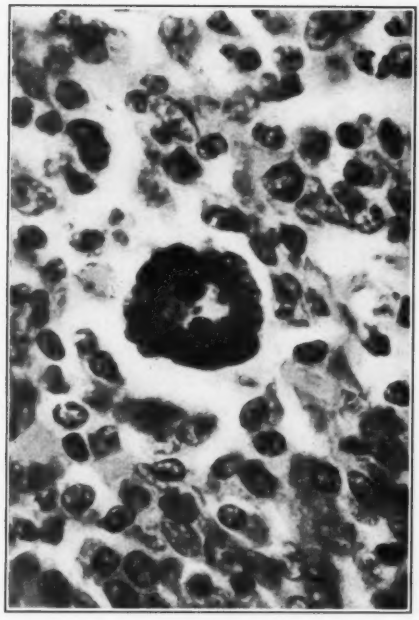

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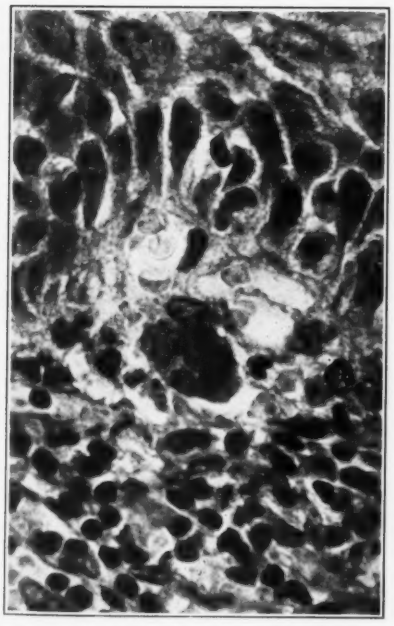

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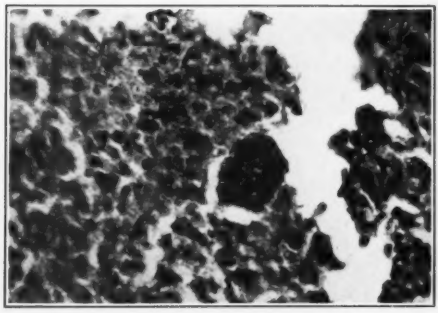

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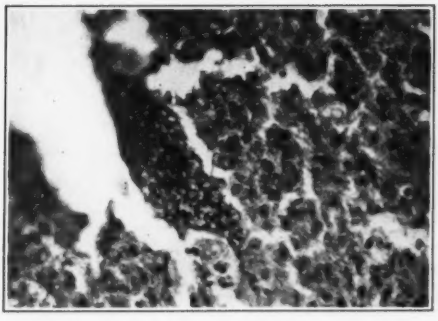

6

Tomlinson

Giant Cells in Tonsils in Chickenpox

TRAUMATIC AUTOTRANSPLANTATION OF SPLENIC TISSUE *

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For several decades it has been known that following severe trauma to the region of the spleen the peritoneum may be found to contain nodules composed of a tissue grossly and microscopically similar to normal spleen. Cases of this type are apparently quite rare, as a survey of the literature shows. Despite this the subject is eminently worthy of study because it involves certain principles of function, growth and transplantation which are among the most fundamental problems of biology.

The earliest well marked case occurring among humans was reported by Faltin.[1]

A boy aged 9 years suffered traumatic rupture of the spleen. Splenectomy was performed. Six years later laparotomy was performed for appendicitis. The peritoneal cavity was found to contain innumerable lentil sized nodules, covered with serosa, and scattered over the large and small intestines. Microscopically these nodules resembled spleen.

Faltin believed that these nodules arose from dormant areas of splenic anlage which as a result of splenectomy had received a stimulus to full development. He felt that the nodules constituted compensation for the splenic tissue removed at operation.

A similar case was reported by Küttner.[2]

An elderly man received bullet wounds of the spleen and colon. Splenectomy and intestinal repair were performed. Four years later the patient died of coronary arteriosclerosis. At autopsy a mass of splenic tissue about 6 cm. in diameter and another about 2 cm. in diameter were found in the left hypochondrium. In addition, innumerable nodules were found over all the intestinal loops. These lesions consisted of splenic pulp, unaccompanied by trabeculae.

This case was repeatedly mentioned in the literature both by Küttner, who performed the original splenectomy, and by Beneke [3] who performed the autopsy.

Oltmanns [4] in a dissertation not accessible to us described a case in which nodules of splenic tissue were found in the peritoneal

* Received for publication June 6, 1939.

cavity of an individual who had previously undergone splenectomy for traumatic rupture of the spleen.

Von Stubenrauch [5, 6, 7] has made a series of clinical and experimental contributions to existing knowledge of the subject. He described a case as follows:

> A man suffered a severe crushing injury of the trunk. At operation, 36 hours later, huge quantities of blood were found in the peritoneal cavity. The spleen was squeezed and broken, the fragments being about 10 cm. apart. After splenectomy and a complicated postoperative course lasting 5 weeks the patient recovered. He subsequently developed intestinal obstruction. Laparotomy disclosed the presence of innumerable nodules ranging from 0.25 to 0.5 cm. in diameter. These nodular lesions contained trabeculae, sinuses and lymphocytes, but were devoid of lymphoid follicles.

Von Stubenrauch was able to implant autogenous splenic tissue in the peritoneal cavity of dogs, cats and rats. He considered splenic tissue highly susceptible to autotransplantation. He believed, however, that splenic autotransplants tend to regress. He stated that splenic function can be compensated for in three ways: (1) by changes in the bone marrow and lymph nodes; (2) by regeneration of the main mass of splenic tissue; and (3) by the formation within the peritoneal cavity of organs which have a structure like that of splenic tissue. To these structures he gave the name "splenoids." This "splenoid" theory seems to have originated with von Stubenrauch and has been accepted by subsequent authors. The existence or non-existence of splenoids is of great importance and will be discussed in the later pages of the present study.

Von Stubenrauch's last article [7] offers these conclusions: (1) in cases of the type previously detailed a traumatic origin of the peritoneal nodules cannot be assumed; and (2) clinical experimental evidence does not warrant the belief that splenic tissue presents any well marked proliferative tendency.

Küppermann [8] states: "It has not rarely happened that in splenectomized persons who for any reason were subjected to subsequent laparotomies the peritoneal cavity has been found to contain certain small formations which on microscopic examination proved to be accessory spleens." This comment accompanies the report of the following case:

> A boy aged 15 years was hurt in a bicycle accident. The spleen was found to be smashed and was accordingly excised. A half year later the patient was

operated on for incisional hernia. The peritoneal cavity contained about 100 brown and red nodules, some of which were about 0.5 to 1 cm. in diameter. On microscopic examination these were shown to consist of normal splenic tissue.

Shaw and Shafi,[9] whose extensive review we have used freely, reported the following case:

An Egyptian male aged 20 years had undergone splenectomy for a traumatic lesion of the spleen. Several years later this patient died in uremia. He was found to have 82 nodules, most of which were in the peritoneal cavity. One nodule was found in the left pleural cavity, 1 on a vertebra, and 1 beneath the capsule of the liver. The nodules contained structures resembling splenic pulp and capsule.

These 6 cases gathered from the literature bear the closest possible resemblance to the following hitherto unpublished cases.

Case Reports

Case 1: A boy aged 6 years was knocked down by a truck. The wheel of the truck struck the left side of the boy's chest and abdomen but did not run completely across the trunk. The family noticed that the patient was pale and very thirsty. About 16 hours after the accident he complained of pain in the upper left abdominal quadrant, which was aggravated by coughing. The patient was brought to the hospital and laparotomy was performed at once, a ruptured spleen being removed.

The spleen weighed 90 gm. It bore a ragged tear extending half way through the organ from about the middle of the anterior margin. Section of the splenic tissue showed no abnormality except for hemorrhage around the laceration.

Subsequent roentgen examination disclosed the presence of a fracture of the clavicle but no other bony abnormalities. A blood transfusion was given and prompt recovery ensued, the hemoglobin increasing rapidly from 55 to 100 per cent. The healing of the wound was delayed by several stitch abscesses.

During the next 1½ years the child enjoyed good health.

At the end of this period, *i.e.* 2 days before his death, he began to complain of cramp-like pain around the operative scar. He refused food and began to vomit. On admission to the hospital 48 hours later he was in extremis; he had poor color, grunting respiration, and a distended tympanitic abdomen. He died 3 hours after admission. The clinical diagnosis was peritonitis secondary to rupture of the appendix.

Postmortem Examination: The changes of greatest interest are in the peritoneal cavity. The peritoneal surfaces bear a little film and there are 200 cc. of amber serous fluid in the peritoneal cavity. Several fibrous adhesions bind the splenectomy wound to various loops of intestine and to the omentum. The largest mass of adhesions is attached to the ileum at a point 62 cm. above the ileo-

cecal junction. The intestines proximal to this point are distended with gas and liquid fecal material; those distal to it appear normal. The intestinal lumen is continuous but the proximal loops pass back and forth beneath the terminal ileum. The last segment immediately proximal to the site of the adhesion is somewhat twisted in its course and bears a red and friable mucosa.

Scattered over the peritoneal surface are several dozen nodules of splenic tissue. These are found on the inferior surfaces of the left dome of the diaphragm and the left lobe of the liver, the anterior surfaces of the stomach, lesser omentum, the transverse colon and right kidney, over the pelvis including the anterior surface of the rectum, and the posterior surface of the bladder. There are also 3 large accessory spleens and several small ones in the omentum. The largest of these measures 2.5 cm. in diameter. The other splenic nodules range from 1 to 12 mm. in diameter. The smaller ones are rounded; the larger ones are flat and 2 to 3 mm. in thickness. All are covered with serosa. Section through the larger nodules shows the structure of a normal spleen with rather irregular capsule and red pulp. Malpighian corpuscles can be distinguished with difficulty.

Microscopic Examination: The splenic nodules have the microscopic appearance of normal spleen, including capsules and malpighian corpuscles. They show the changes characteristic of acute splenic tumor. The malpighian corpuscles are large, and around each is a zone containing many red cells and a few polymorphonuclear leukocytes. One nodule attached to the colon contains iron pigment within phagocytes. These splenic nodules obtain their blood supply from the adjacent tissues.

Sections were also taken from the heart, lungs, liver, pancreas, adrenals, kidneys, prostate, intestines and lymph nodes. These showed no changes except those indicated in the anatomical diagnoses.

Anatomical Diagnoses: Traumatic rupture of spleen; operative splenectomy; adhesions between ileum and operative wound; intestinal obstruction due to postoperative adhesions; gangrene of ileum; gangrene of lung due to aspiration of vomitus; autotransplantation of splenic tissue on peritoneal surfaces of liver, diaphragm, intestine, omentum, kidney and pelvis; and accessory spleens.

CASE 2 *: A boy aged 12½ years sustained a severe blow on the abdomen while coasting. He then walked about a quarter of a mile to the hospital. He presented the complaint of pain in the left upper abdominal quadrant and in the left shoulder. These symptoms were aggravated by deep breathing. The patient was found to be a well developed boy, pale and ashen. The pulse was small, its rate 132. There was mild tenderness on deep pressure over the left upper abdominal quadrant.

The patient became increasingly pale and dyspneic. At operation, 3 hours after the original injury, about a liter of partly clotted blood was found in the peritoneal cavity. The spleen was deeply lacerated but not otherwise abnormal. It was excised, after ligation of the pedicle. About 450 cc. of blood removed from the peritoneal cavity was strained through gauze, citrated, and injected intravenously. The postoperative course was complicated by an infection of the operative wound and by phlebitis.

Eight years later the patient died of appendicitis and multiple peritoneal abscesses.

Postmortem Examination: The autopsy disclosed, in addition to these lesions, approximately 80 nodules ranging from about 1 mm. to 3 cm. in diameter. These were situated in the peritoneum, omentum and diaphragm, and along the greater curvature of the stomach. There were numerous adhesions about the splenic bed. The splenic artery and vein had become recanalized and were found to have sent off branches to some of the nodules.

Microscopic Examination: Sections of 10 nodules were available for study and may be described collectively. The capsules are found to be thin. In most instances typical trabeculae are absent. In 1 nodule a band of connective tissue extends downward from the capsule into the pulp and contains a relatively large blood vessel; the pattern is that of a trabecula. For the most part the nodules are composed of tissue which in appearance and arrangement resembles the red pulp of spleen. In one instance a small group of lymphocytes resembles a crude follicle. The sinuses of the pulp are readily discerned as almost all are engorged. Scattered siderophages are encountered.

A minute nodule found in the subserosa of the stomach differs from the foregoing in certain details. This nodule is encased in a dense collagenous capsule. The pulp consists of lymphoid tissue, with sinusoids and one well marked follicle containing an arteriole. In a section stained with silver an argyrophilic reticulum is dem-

* This case is reported through the kindness of Dr. Paul Klemperer of the Mount Sinai Hospital.

onstrated. The relation of the nodule to the serosa is not clearly determinable from this section.*

In view of the fact that blood removed from the peritoneal cavity at the time of splenectomy had been injected intravenously, it is interesting to note that there were no splenic nodules in the lungs.

CASE 3†: This patient was severely injured in an automobile accident several years ago. As a result of this accident she has a scar in the skin of the left hypochondrium. A pelvic operation performed several years after the original trauma revealed the presence of nodules scattered over the intestines. A nodule removed for biopsy was shown to consist of tissue resembling spleen. At the present time it is still not known whether splenectomy was performed in this case.

In each of the 10 foregoing cases there was a clear history of trauma.

Completeness requires the mention of certain additional cases in which there was no definite history of antecedent trauma. The first of these is Albrecht's widely quoted case.[10]

A man aged 25 years died of nephritis. Scattered throughout the peritoneal cavity, omentum, diaphragm and pelvis were about 400 nodules which Albrecht regarded as accessory spleens. The principal spleen was about 2.5 cm. in diameter and was adherent to the diaphragm and omentum. At its lower pole there were several packets of partly adherent accessory spleens. The left kidney was flattened, shrunken and scarred.

In his comment on this case Albrecht first refuted Toldt's hypothesis that the coelomic epithelium of the mesogastrium might possess spleen-forming potentialities at many points. Such an explanation would not account for the lesion of the left kidney. Albrecht preferred to believe that during intrauterine life a strong and presumably mechanical disturbance had acted upon the region of the spleen and left kidney. As a result, the kidney was almost completely destroyed, while the splenic anlage was broken into countless pieces, which were scattered over the peritoneal cavity

* During a recent visit to a neighboring republic one of us (S. J.) saw a patient whose history was almost identical with that of the patient just described. A young male, evidently about 17 years of age, had undergone splenectomy for traumatic rupture of the spleen. Several years later he was operated on for appendicitis. Innumerable nodules resembling small spleens were found in the peritoneum and on the intestines. It is to be hoped that this case will ultimately be published in detail by the patient's physicians.

† Through the courtesy of Dr. Henry Horn the authors have recently learned of this case which is to be published by Dr. J. H. Buchbinder and Dr. C. J. Lipkoff of the Knickerbocker Hospital.

and in the omentum and became implanted there. These implants would of course be carried along in the wake of subsequent embryonal development and would suffer the same alterations of position and arrangement as the omental and peritoneal reflections. Albrecht abstained from stating specifically that the abnormal process was due to trauma. He judged that the cause of the condition was unknown but was presumably mechanical in nature.

Albrecht's case has been mentioned repeatedly in the literature. It was discussed at a meeting of the Gesellschaft deutscher Naturforscher und Ärzte. On this occasion Beneke [3] presented Küttner's aforementioned frankly traumatic case. Beneke remarked that in his opinion Albrecht's case may have been traumatic in origin. In the discussion Sternberg [11] said that in Albrecht's case trauma had been considered but that no definite evidence thereof had been found. Paltauf [12] stated that he also was familar with Albrecht's case and believed the cause to be trauma.

The case reported by Schilling [13] (quoted by Shaw and Shafi [9]) likewise presents no definite history of trauma.

The patient was a woman aged 47 years who had died of carcinoma of the uterus. In the abdominal cavity there were 42 nodules ranging from about 1 mm. to 2 cm. in diameter. The main spleen was deeply involved in adhesions, sharply angulated, and divided into three lobes by deep incisures. The left kidney measured only 2 by 1 by 0.2 cm. and was deformed. Almost all the nodules were found to have normal splenic structure.

After an extensive discussion of the embryology of the peritoneum Schilling concluded that some unknown principle, acting during intrauterine life, had scattered part of the splenic anlage over the peritoneal cavity. This unknown agent was considered insufficient to stop the further development of the principal spleen but had impaired the progress of the left kidney.

The following case was reported by Tedeschi.[14]

A girl aged 15 years died of meningitis. The spleen was found to be ectopic and somewhat atrophied, and bore many depressed scars. The transverse colon was displaced obliquely toward the left. The spleen was situated deep in the left side of the peritoneal cavity. Along the splenic vessels there were about 50 accessory spleens, and 2 more were found in the gastrocolic omentum. There were many lymphoid formations in the liver.

A similar case was reported by Jolly.[15]

A girl aged 15 years had vague abdominal pains and nausea and was thought to have appendicitis. At operation the peritoneum was found to contain huge numbers of round and ovoid nodules ranging from 0.1 to 0.5 cm. in diameter. These nodules contained well marked malpighian corpuscles and were believed to consist of splenic tissue. The condition of the main spleen was not ascertained. The history made no mention of trauma.

Of these cases all but 4 present a clear history of antecedent trauma. In 4 cases (Albrecht,[10] Schilling,[13] Tedeschi,[14] and Jolly [15]) the clinical histories make no mention of trauma. The facts which these cases provide are fortified by certain observations derived from a study of lower animals.

According to Ceresole,[16] Zambeccari [17] in 1680 was the first to observe nodular formations after experimental splenectomy. From the quotation given by Ceresole there is some doubt that the nodules described by Zambeccari were similar to the nodules which form the subject of the present discussion. More precise descriptions were given by many more recent authors, such as Tizzoni,[18–23] Abelous, Argaud and Soula,[24] Amormino,[25] and others.

Guerrini [26] reported the case of a dog aged 8 years. Ten months prior to its death this animal had suffered a severe physical injury which had been followed by prolonged illness and recovery. Ultimately the animal died of a diarrheal disease. Nodules of splenic tissue were found widely scattered in the peritoneal cavity. The spleen contained a hemorrhagic cyst but had no wound on its surface. Consequently Guerrini concluded that the numerous splenic nodules were not implants. He inferred that the splenic cyst had produced splenic insufficiency; as a result of this deficiency a swarm of preëxisting accessory spleens had been stimulated to hypertrophy.

The present authors disagree with Guerrini's interpretation. The so-called hemorrhagic cyst was very probably the result of laceration of splenic pulp — the so-called incomplete rupture of the spleen, the pathogenesis of which is well described by Englmann and Hitzler.[27] It is not unlikely that a splenic scar was present near the cyst but was small and escaped detection. The peritoneal nodules thus could easily have been produced by implantation of splenic tissue scattered at the time of the splenic injury. Moreover, in the recent experiments of Bloom and Taliaferro [28] less than 1 per cent of experimental infarcts of the spleen

were followed by permanent scarring. It is therefore possible that in Guerrini's case the animal originally suffered an incomplete rupture of the spleen and splenic pulp was scattered in the peritoneal cavity. The deeper portions of the splenic rupture then healed incompletely and formed a cystic and hemorrhagic lesion, while the surface of the spleen regenerated completely.

The coexistence of blood cysts of the spleen and intra-abdominal nodules was also reported by Binet.[29]

Tizzoni,[18] who did much of the important earlier work in this field, reported that in 1 dog he found 262 nodules of splenic tissue in the peritoneal cavity. The main spleen was extensively scarred by "chronic interstitial splenitis."

Professor Jarmai [30] of the veterinary college at Budapest reported the case of a terrier which died a few days after an injury. The spleen was found to be broken completely in half; the larger half bore two scars. More than 400 nodules resembling spleens were found growing in the peritoneal cavity. Subsequently it was learned that in previous years the dog had suffered two severe injuries, including a fall from a window.

Jarmai mentions more briefly the case of another dog which had had an injury 1 year before death and which was ultimately found to have 483 accessory spleens. Köves [31] (quoted by Jarmai) reported a less conclusive case occurring in a pig. This paper has not been available to us.

By reason of their uniformity the foregoing cases are readily summarized. In certain individuals who had undergone splenectomy for traumatic rupture of the spleen, the peritoneal cavity was subsequently found to contain a smaller or larger number of nodules composed of a tissue closely resembling spleen. Four additional cases presented nodules of this type without data as to previous trauma; in 3 of these 4 cases the main spleen was small and shrunken or distorted; in 2 of the 4 cases the left kidney was similarly affected. Parallel observations in animals are also cited.

In explanation of these phenomena several different hypotheses suggest themselves. We may at once dismiss the possibility that the nodules *precede* the splenic trauma and hence are unrelated thereto. This view — which at all events does not apply to the cases in humans — depends on the fact that small animals, espe-

cially dogs, are occasionally found to have unexplained splenic tissue in the peritoneal cavity. This tissue, in accordance with arguments to be presented (*vide infra*), is more probably attributable to the myriad injuries which small animals inevitably suffer.

A second hypothesis proposes that the nodules are formed by the enlargement of preëxisting lymphoid tissue or splenic anlage. It has been thought that splenectomy might stimulate such structures to proliferate and to form nodular masses.

This opinion was held by many of the earlier students of the problem, such as Foà,[32] Faltin,[1] and Capelli [33]; it receives its support in part from analogy. Thus it is stated that splenectomy may be followed by general enlargement of lymph nodes.[34] One might also cite the fact, mentioned by Schmidt,[35] that in mice splenectomy is followed by the development of nodular formations (lymphomas?) in the liver. The observations of De Kock [36] in ruminants are likewise of interest in this connection.

A serious objection to the "compensatory hyperplasia" hypothesis is the fact that nothing recognizable as splenic anlage or lymphoid tissue is known to occur in such places as the intestinal subserosa or the diaphragm, which are among the commonest sites of the nodules. It must also be remarked that *the occurrence of widely disseminated splenic nodules in any considerable number has never been reported after splenectomy in cases of non-traumatic disease of the spleen. This suggests that the determining factor is not splenectomy but trauma.*

Specific data confirming this statement have been obtained from the autopsy files of the Presbyterian Hospital and of the Babies Hospital. In the last 2000 autopsies at the Babies Hospital there were 9 cases in which splenectomy had been performed for various reasons. The interval between splenectomy and death ranged from a few hours to 9 years. The only case in which the operation had been performed because of traumatic rupture of the spleen was that reported in this paper (Case 1) and it was likewise the only case in which there were nodules widely spread over the peritoneal surfaces. In 2 other cases there were accessory spleens in the usual site in the fat near the bed of the spleen. During the last 10 years there have been 2605 autopsies at the Presbyterian Hospital; there had been a previous splenectomy in 16 of

these cases; the interval between operation and death varied from 2 weeks to 15 years. In no instance in the Presbyterian autopsy series was splenectomy performed because of traumatic rupture, and in none of these cases was the splenic tissue found elsewhere than in the bed of the spleen. Accessory spleens were found in 3 cases.

A third hypothesis is of even greater theoretical interest. This is the dictum that the nodules are formed by the peritoneum, perhaps under the stimulus of splenectomy.

It has been difficult to discover who first presented this concept. Apparently one of the earliest was Toldt, whose opinion Albrecht [10] quotes (without adequate bibliographic reference) and rejects. Toldt is quoted as having said that the splenic anlage arises by a special alteration of the coelomic epithelium. This alteration ordinarily occurs in a single area but under exceptional circumstances might occur in a number of unconnected foci and thereby generate any given number of unconnected splenic nodules. As has been stated, Albrecht felt that such an explanation could not account for concomitant lesions of the left kidney; he assumed instead that during fetal life a strong and presumably mechanical disturbance had struck the region of the spleen and left kidney, scattering the anlage of the former and damaging the anlage of the latter. Albrecht's explanation must be considered a closer approximation to the truth.

Another early study of the subject was that made by Griffini and Tizzoni [37] in 1883. These authors observed that partial splenectomy in dogs was followed by the development of nodules in the peritoneum. They concluded as follows: "These observations confirm once again that the omentum and the folds of the peritoneum have the property of giving rise to 'new productions' of splenic parenchyma." Similar opinions were expressed independently by Tizzoni.[23] Certain serious flaws in the work of Tizzoni have been pointed out by Meyer.[38]

Faltin's [1] views have already been mentioned. This observer felt that splenectomy stimulated the peritoneum to regain its alleged former power of creating splenic tissue. In other words, dormant areas of splenic anlage were awakened to compensatory activity.

It is in the writings of von Stubenrauch [5, 6, 7] that the theory of

peritoneal origin reaches its fullest development. Von Stubenrauch stated that after excision of the spleen restoration occurred in three ways: (1) by changes in the bone marrow and lymph nodes; (2) by regeneration of the spleen; and (3) by the new formation in the peritoneum of organs resembling spleens. To the latter, which are the nodules under discussion, von Stubenrauch gave the name of "splenoids." Accordingly the entire conception of the peritoneal genesis of splenic nodules can be not inappropriately designated "the splenoid theory."

Von Stubenrauch performed a large number of successful implantations of autogenous splenic tissue in various experimental animals. These experiments resulted in the formation of nodules resembling spleens. Von Stubenrauch concluded that implanted splenic tissue ultimately regressed. He felt that the nodules found in the aforementioned human cases had been created by the peritoneum. Von Stubenrauch's views have been handed down in the literature [39] and seem to have acquired a permanent niche. In Herfarth's review [40] the splenoid theory is considered "possibly correct but by no means conclusively proven." The most recent general treatise on the spleen, that by Klemperer,[41] states: "Clinical and experimental observations show that the loss of splenic tissue can be compensated for in various ways (von Stubenrauch, 1920; Herfarth, 1926): (1) by hypertrophy of splenic tissue left behind at operations; (2) by hypertrophy of accessory spleens (Morrison *et al.*, 1928); (3) by autotransplantation of pulp particles (Kreuter, 1920); and (4) *by formations of spleen-like structures originating from the peritoneum* (splenoids)." (Italics ours.)

This abstract is given in full because it shows that the "splenoid" theory has been accepted by eminent authorities and has won a degree of credence which the facts may or may not warrant. The doctrine that spleen-like structures may be formed from the peritoneum is of fundamental biological importance and deserves complete reinvestigation. Studies now in progress by one of us (S. J.) will, it is hoped, contribute new data toward the elucidation of this problem.

From these considerations it is clear that the "preëxisting nodule hypothesis," the hypothesis of compensatory hyperplasia, and the so-called "splenoid theory" all present certain virtues and

certain inadequacies. One theory remains to be discussed — that the nodules represent implants of splenic tissue scattered throughout the peritoneal cavity as a result of laceration or rupture of the main spleen.

Autoplastic transplantation of splenic tissue has been successful in the hands of many experimenters (Ehrhardt,[42] Manley and Marine,[43] Marine and Manley,[44, 45] von Stubenrauch,[5] Kreuter,[46] Koppányi,[47] Jolly and Lieure,[48] Putschar,[49] and Fujigaki[50]). Genetic aspects of splenic transplantation have been studied by Bittner[51] and by Little and Johnson.[52] Manley and Marine[43] found that spleen autotransplants with considerable difficulty, as compared with thyroid, parathyroid, ovary or adrenal cortex. Marine and Manley[45] found that in rabbits removal of the spleen provides a powerful stimulus to the growth of transplants; this opinion is not universally accepted. Silberberg[53] made autotransplants in guinea pigs and observed that lymphoid and hematopoietic tissue in general have no marked resistance to transplantation.

Perla and Marmorston-Gottesman[54] observed a striking difference in the regenerative capacity of autoplastic splenic transplants in rats as compared with rabbits. Marine and Manley[44] had shown that in the adult rabbit splenic autotransplants would grow in the absence of the main spleen but frequently remained small or even were resorbed. Perla and Marmorston-Gottesman found that in the rat splenic autotransplants would grow even in the presence of the spleen and in its absence would undergo marked hypertrophy. Detailed microscopic studies of autoplastic splenic transplants have recently been published by Perla.[55]

The extensive experiments of von Stubenrauch have been discussed above. He succeeded in obtaining splenic autotransplants yet felt that these were subject to regression and that permanent nodular formations were produced by the peritoneum.

Ehrhardt,[42] using rabbits, obtained successful autoplastic transplants of pieces of spleen and also of whole spleen.

The experiments of Kreuter[46] require detailed consideration. Working with rhesus monkeys, Kreuter found that total splenectomy produced no compensatory formations. Partial splenectomy produced a few nodules resembling accessory spleens. If the spleen was excised and its pulp was smeared all over the peri-

toneum, widely scattered nodules were later found. Kreuter concluded that these nodules could be explained only as the result of trauma and had nothing to do with compensatory hyperplasia or atavism.

Despite Kreuter's controlled and seemingly decisive experiments, Herfarth [40] in a subsequent review concluded that the possibility of autotransplantation (in humans) had not been established beyond cavil but was plausible. This conclusion is a good example of the unwillingness which many of the earlier authors have manifested toward accepting the existence of spontaneous autotransplantation. Herfarth's attitude toward the splenoid theory has been discussed above.

Even more significant was the work of Putschar.[49] This experimenter used the rat, which does not often have accessory spleens. He placed only 3 or 4 implants in each animal. These implants were relatively large (3 to 5 mm.). Some were inserted intraperitoneally, others subcutaneously. The resultant nodules resembled spleens and contained follicles. In 12 rats all implants but 2 were accounted for. Putschar concluded that the nodules could not be regarded as either enlarged hemolymph nodes or as newly created peritoneal "splenoids," since such an explanation could not account for the nodules that had been formed in the subcutaneous tissues.

In view of the work of Kreuter and of Putschar the splenoid theory would seem greatly weakened. Further studies are clearly necessary. Even now it is scarcely possible to avoid the conclusion that the intraperitoneal nodules found after splenectomy in cases of splenic rupture are due to the scattering of particles of pulp throughout the peritoneal cavity. Such scattering is undoubtedly assisted by the profuse hemorrhage which accompanies splenic rupture. This explanation has the merit of simplicity and the support of experimental observation. It obviates the necessity of von Stubenrauch's awkward and otherwise poorly supported assumption that the peritoneum is capable of manufacturing splenic tissue.

Summary and Conclusions

1. Two cases are presented in which individuals who had undergone splenectomy for splenic rupture were subsequently found

to have numerous nodules of spleen-like tissue scattered throughout the peritoneal cavity.

2. Mention is made of 8 additional cases gathered from various sources, and of 4 cases in which no definite history of trauma was available. In 2 of these the spleen and left kidney were shrunken, scarred and distorted. Several analogous cases in animals are quoted from the literature.

3. The experimental evidence is summarized and it is shown that splenic tissue is susceptible to autoplastic transplantation.

4. The "splenoid" theory is described and is shown to be a gratuitous assumption inadequately supported by evidence.

5. It is concluded that the aforementioned nodules, found in the peritoneum and omentum of individuals who have previously undergone splenectomy for traumatic rupture of the spleen, are due to autoplastic transplantation of particles of spleen torn loose from the main body of splenic tissue and disseminated in part by the aid of hemorrhage.

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DESCRIPTION OF PLATES

PLATE 88

FIG. 1. Case 2. Splenic implants on the anterior wall of the stomach.

FIG. 2. Case 1. Splenic implants on the pelvic peritoneum.

FIG. 3. Case 1. Accessory spleens in the omentum.

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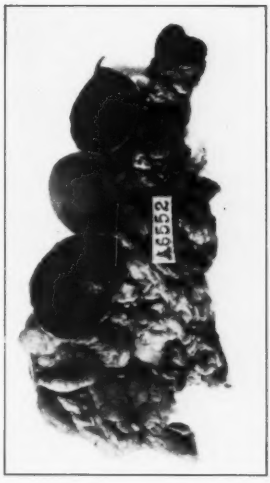

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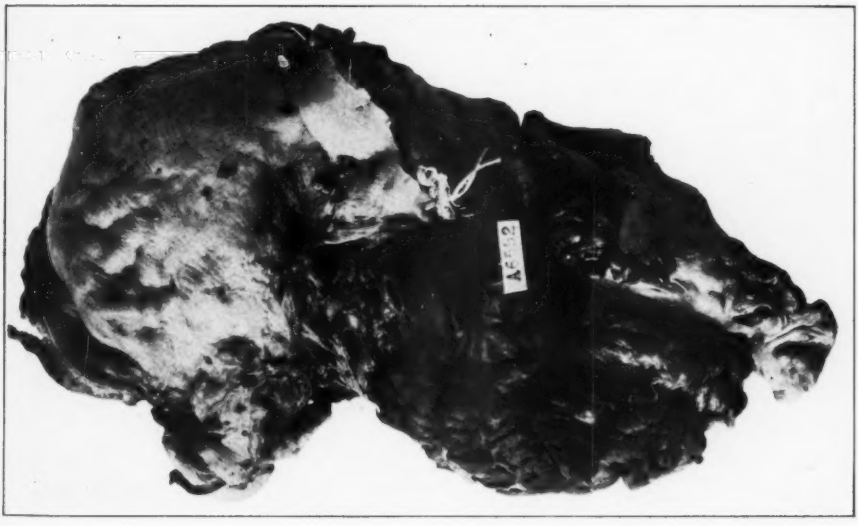

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Plate 89

Fig. 4. Case 1. Splenic implant on the diaphragm.

Fig. 5. Case 1. Malpighian corpuscle in a splenic implant.

Fig. 6. Case 2. Nodule in the wall of the stomach.

Fig. 7. Case 2. The nodule illustrated in Fig. 6 is shown here in greater magnification. Part of a malpighian follicle and its arteriole are shown.

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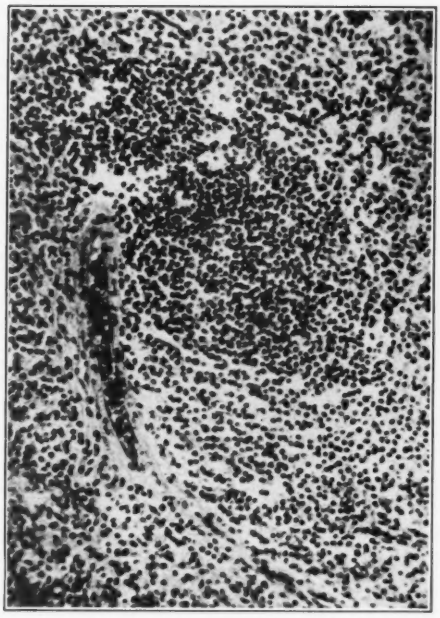

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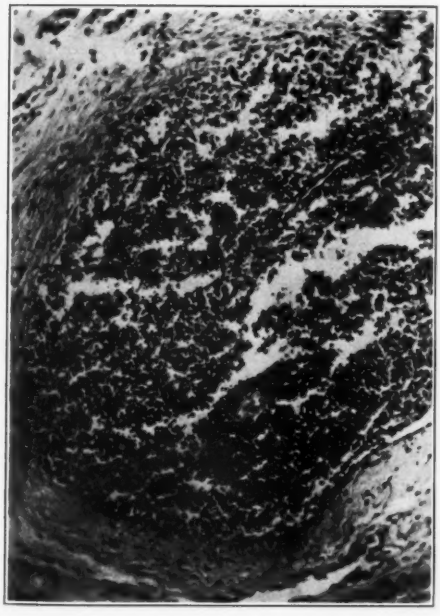

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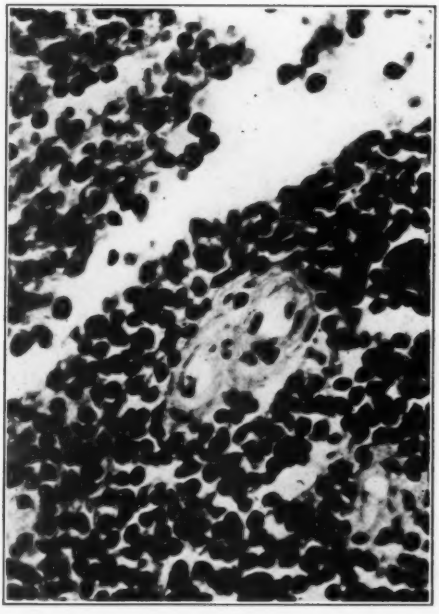

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Jarcho and Andersen

Traumatic Autotransplantation of Splenic Tissue

A COMPARISON OF THE EFFECTS OF ANTERIOR PITUITARY HORMONE ON SKELETAL TISSUES OF YOUNG AND MATURE GUINEA PIGS *

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Anterior pituitary hormone exerts a growth-promoting effect on the euhyaline cartilage of immature guinea pigs and also produces certain degenerative changes in this tissue.[1, 2] These effects were observed in experiments in which anterior pituitary of cattle was implanted, as well as in those in which injections of acid extract of this gland were given. After the demonstration of this action of anterior pituitary hormone on the skeleton of immature animals, it appeared to be of interest to investigate the response of mature skeletal tissues to the same stimuli and, if possible, to correlate them with the changes which normally take place in these tissues with advancing age, particularly since Loeb and his coworkers [3, 4, 5] have shown that hormones may call forth alterations in the stroma of the thyroid and the mammary glands of guinea pigs and of the vagina and the uterus in mice.

MATERIAL AND METHODS

Thirty-eight guinea pigs were used in these experiments which were divided into two series. The first series consisted of 16 animals weighing about 400 gm. at the beginning of the experiment. Seven of these animals received 2 cc. each of acid extract of anterior pituitary gland of cattle daily for periods of 4, 15, 30 and 60 days. Two animals received implants of one-half of a gland of fresh anterior pituitary of heifer on 2 consecutive days and were sacrificed on the 4th day. Two guinea pigs received daily implants of one-half of a fresh gland of anterior pituitary of heifer on 4 days and were killed on the 5th day. Two animals also received four implants of one-half of a gland of anterior

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pituitary of heifer on 4 consecutive days after the glands had been subjected to immersion in urea for 15 hours and in glycerine for 24 hours previous to implantation [6]; these animals were also killed on the 5th day. Three guinea pigs of corresponding weight served as normal controls.

In the second series, consisting of 22 guinea pigs, with an initial weight of 750 to 900 gm., larger amounts of pituitary substance were administered, in accordance with the greater weight of the animals. Thirteen animals were injected daily with 4 cc. of acid extract of anterior pituitary gland of cattle intraperitoneally for periods of 4, 5, 6, 7, 10, 14, 30, 45 and 60 days. Two additional animals received daily implants of one-fourth of a gland of fresh anterior pituitary of heifer on 2 consecutive days and were killed 2 days after the second implant had been made. Two guinea pigs received the same amount of fresh anterior pituitary gland daily on 4 consecutive days and were sacrificed on the 5th day. Five animals served as normal controls.

At autopsy the bones were removed for study. Since in the heavy animals our usual technic of incomplete decalcification proved to be a very slow process, some of the bones were decalcified completely in 5 per cent nitric acid. In all other respects the technic employed was the same as in our former investigations.[2]

Observations

The animals weighing 400 gm. stood the injections of acid extract of anterior pituitary gland well, without showing any marked impairment of their general health, although within the first 10 to 15 days a loss in weight amounting to approximately 10 per cent of the initial weight occurred. But in the animals that were kept alive for periods of 1 and 2 months the weight was soon restored to the initial level, and during the 2nd month of the experiments a gain in weight of about 20 per cent was noted.

The experiments in the second series, however, presented some difficulties, inasmuch as the animals weighing 750 to 900 gm. exhibited a very low resistance to the extract. Their weight fell rapidly, sometimes as much as 15 to 20 per cent within the 1st week of treatment. Some animals had, therefore, to be sacrificed prematurely. Those that survived continued to lose weight,

and at the end of the experiments losses of 35 to 45 per cent were noted.

Microscopic Examination

I. Normal Animals

Cartilage: (*A*) In healthy guinea pigs weighing about 400 gm. and approximately 3 to 4 months old the epiphyseal line was still patent. The cartilaginous ground substance was dense and fibrillar; in some instances collagenous formations had been laid down in this ground substance. The arrangement and distribution of the rows of cartilage cells were less regular than in immature animals. The various cell types, however, were still readily recognizable. The resting cartilage cells were small and flattened, the columnar cartilage cells were fairly numerous, and the replacement of the hypertrophic cartilage cells by bone proceeded in the normal way. In some instances there was evidence of beginning ossification of the epiphyseal line; degenerated cartilage cell columns were replaced by localized wedge-like bony plugs containing remnants of former cartilage cells and horizontal cracks; or here and there a cartilage cell was seen being directly converted into an osteocyte.

In the cartilaginous covering of the joint the sliding, transitional and pressure zones were composed of their characteristic cellular constituents. The layer of the hypertrophic cartilage was almost ossified. In the cartilaginous part of the ribs some hyalinization of the intercartilaginous matrix was observed; the cartilage cells, particularly in the central portions, revealed a slight tendency to undergo either atrophy or hypertrophy, both of these processes being followed by degeneration and solution; but usually only a single cell or isolated cell groups were affected.

(*B*) In normal healthy guinea pigs weighing about 800 gm. the condition of the epiphyseal cartilage was influenced by the age of the animals. In some cases the epiphyseal line of the tibia was very narrow, but it still contained remnants of columnar and especially of hypertrophic cartilage cells which underwent calcification and ossification, and considerable amounts of stroma consisting of collagenous or osseous material were seen. In some instances bundles of parallel thick fibers appeared in the substance separating the cartilage cells, a condition typical of the so-called

asbestos transformation. This condition was found when the guinea pigs were approximately 8 to 12 months old. However, in animals that were in the 2nd or 3rd year of life the upper tibia showed only islands of euhyaline cartilage cells, which indicated the site of the original zone of ossification. As a rule progressive ossification and infiltration by lymphoid marrow had led to a fusion of the diaphysis and epiphysis. In circumscribed, centrally located areas the cartilage of the ribs had undergone retrogression; it was liquefied and cysts had formed in some instances; or the retrogressed parts had been partly replaced by hyaline, calcified or osseous material. The older the animals, the more advanced the retrogressive changes. The demarcation between the cartilaginous and osseous parts of the ribs was sharp. There was no evidence of an invasion of the cartilage by bone marrow. The cartilaginous ground substance of the covering of the joint was acidophilic and hyalinized. The cartilage cells were in a resting stage. Occasionally, in very old guinea pigs, some cells tended to undergo degeneration.

Periosteum and Bone Marrow: With progressing age the periosteum and the interstitial connective tissue of the bone marrow increased in amount and became denser. The fibers became hyalinized. In some circumscribed areas the lymphoid cells of the marrow, particularly in the epiphysis, had been gradually replaced by fat tissue. In the diaphysis these alterations were less accentuated. Cells acting as osteoblasts and as osteoclasts were present, indicating that appositional and resorptive processes still took place in mature and in old animals.

II. Implants of Anterior Pituitary Gland and Injections of Extract of Anterior Pituitary Gland of Cattle into Young and Old Mature Guinea Pigs

(A) Animals Weighing 400 Gm. at the Beginning of the Experiment.

Cartilage: After intraperitoneal injections of 2 cc. of acid extract of anterior pituitary gland of cattle daily on each of 4 consecutive days, processes of softening, swelling, vacuolization and liquefaction of the cartilaginous ground substance of the epiphyseal line were observed. The fibrils were loosened and torn apart. Owing to deposition of calcium in and around such de-

generated areas the epiphyseal zone assumed a darker stain with hematoxylin. The resting and particularly the columnar cartilage cells underwent retrogressive changes, the outlines of the cells becoming more and more indistinct and the nuclei pyknotic. Ultimately these cells disintegrated or became calcified. In some cases only single cells or individual cell groups degenerated; in other instances larger areas of the epiphyseal line were destroyed. Side by side with these degenerative alterations of the cartilage cells growth processes took place. The resting and the columnar cartilage cells proliferated by way of amitosis or, here and there, by mitotic division. The degenerative changes were essentially the same as those seen in guinea pigs weighing 180 gm. injected with 1 cc. of acid extract for 4 consecutive days, although they were somewhat more extensive. On the other hand, the tendency to calcification and proliferation seemed to be less marked here than in the immature guinea pigs. At this early stage there was only a suggestion of the formation of incubator capsules in the chondrophyte; retrogressive and proliferative processes in the ribs and joints were slight or lacking altogether. The lesions obtained after four implantations of one-half of a gland of anterior pituitary of heifer on each of 4 consecutive days were comparable to those seen after four injections of extract, but the effects of two implantations of one-half of a gland of heifer were less pronounced. No difference in the action of fresh glands and of those in which the thyroid-stimulating hormone and the atresin had been destroyed by the *in vitro* treatment of the glands with urea and glycerine [6] could be established.

With an increasing number of injections the degenerative changes in the cartilaginous ground substance receded gradually, whereas the proliferative processes became more marked. After 1 month of treatment the epiphyseal cartilage cells were arranged in regular rows and were more numerous than in the control animals. The epiphyseal line was still patent; it maintained its normal structure and there was none or only a slight evidence of osseous closure of the epiphyseal disc, as seen ordinarily at this stage. In the chondrophyte, cartilaginous brooding capsules appeared. In the joint the cartilaginous covering was thickened; the cartilage cells of the transitional and pressure zones, in which ordinarily the long axis was in a horizontal direction, assumed a

perpendicular arrangement and took on a darker stain with hematoxylin; they proliferated freely by means of amitotic division. Simultaneously the cytoplasm became lighter and enlarged and the nuclei were surrounded by an alveolar space. In some cells atrophy had taken place; in others, and in particular in hypertrophied cells, the nuclei had become pyknotic and the cytoplasm had liquefied and finally dissolved. In addition, capillary loops from the bone marrow perforated the bony border lamella and penetrated into the cartilage (Fig. 1). The ground substance was diminished and hyalinized. In the ribs, particularly in their central portions, similar changes occurred, leading to the formation of minute gaps in the cartilage or to the appearance of hyalinized asbestos fibers.

After 2 months of treatment closure by ossification of the epiphyseal zone had set in, but the epiphyseal line was still wider and the number of the cartilage cells greater than normal. In the joint degenerative processes of the cartilage cells became more accentuated and minute irregularities of the joint surface were noted. In the ribs larger cysts were seen filled with mucoid and amorphous material. The line of demarcation between cartilage and bone, however, was sharp.

Periosteum and Bone Marrow: The condition of the osseous tissue was determined by the behavior of the cartilage and the connective tissue in the periosteum and the bone marrow on the one hand, and the balance between appositional and resorptive processes in trabeculae and the compact bone on the other. In the connective tissue a loosening, swelling and tearing apart of the fibrils took place in the early stages. Some reticulum cells of the bone marrow enlarged and assumed a spheroidal and later a polyhedral shape. They appeared honey-combed, the vacuolar spaces being filled with a pale staining clear material (Fig. 2). These cells in which the nuclei were pushed toward the periphery, because of increased cellular pressure, resembled xanthomatous cells and showed all transitions between the latter and ordinary reticulum cells or typical fat cells. They were arranged in islands.

Connective tissue cells along the compact bone and the trabeculae became converted into ameboid epithelioid cells; they were arranged either in a bead-like manner, apparently participating

in the apposition of bone, or underwent fusion and formed multinucleated giant cells. The connective tissue cells, particularly in the subepiphyseal layer of the bone marrow, proliferated and took part in the formation of a fibrotic collagenous tissue which replaced the ordinary lymphoid structure. A similar proliferation in the periosteum led to a thickening of the latter tissue and to the production of precartilage and typical cartilage cells. Following the administration of anterior pituitary extract over a period of from 1 to 2 months, an extensive layer of cartilage cells in the periosteal tissue, situated in the middle and in the upper third of the tibia, was found, and in some places hypertrophied cartilage cells invaded the bony substance. This cartilage underwent hypertrophy and retrogression similar to the cartilage in other regions of the skeleton.

(*B*) *Animals Weighing 800 Gm. at the Beginning of the Experiment.*

Cartilage: The cartilage cells of the joints and of the ribs responded more readily and intensely than the epiphyseal cartilage, which reacted only if large amounts of the hormone had been administered over long periods of time. These changes consisted of a slight hypertrophy and increase in the number of the cartilage cells, mainly in the chondrophyte and in the lateral parts of the epiphyseal zone, which occurred even in instances in which the epiphyseal line was almost completely ossified. An examination of joints and ribs furnished further information as to the action of the hormone. After four implants, or after four injections of the extract, the cartilage cells of the joint, which under normal conditions were resting, proliferated very slightly. The fibrils of the cartilaginous ground substance were loosened and swollen. With increasing doses they disintegrated. The growth processes in the cartilage cells became more accentuated. After 2 to 4 weeks of administration of the extract the cartilaginous covering of the joint was thickened. The cartilage cells of the transitional and pressure zones multiplied and four or more cells were surrounded by one capsule. Subsequently the proliferating cartilage cells underwent hypertrophy. The more the cells enlarged, the more indistinct became the structural details of the nucleus, which finally disintegrated and was destroyed; degenerative changes (karyor-

rhexis and karyolysis) predominated over the growth processes. In some instances the polyhedral cartilage cells assumed a spindle shape and resembled ordinary fibrocytes. The connective tissue cells of the synovial membrane participated in the proliferative processes. In the ligaments, near their insertion, where under normal conditions cartilage cells are found occasionally, an increase in number and size of these cells was observed. The latter likewise underwent retrogressive changes. After 2 months of injections of the extract circumscribed necrotic areas (Fig. 3) and ulcerations of the surface of the joint (Fig. 4) were seen. In some places a pronounced overgrowth of the cartilage cells was present. This pathological tissue, however, showed a marked tendency to disintegration. Degenerated masses accumulated in irregular clumps and advanced toward the surface of the joint where they were cast off. Capillary loops from the bone marrow and the synovial membrane invaded the newly formed tissue. Due to a combination of the proliferative and retrogressive changes, villi or adhesions between the synovial membrane and the cartilaginous covering of the joints were produced (Fig. 5). With increasing amounts of extract, both proliferation and degeneration of the cartilage of the ribs became proportionately more pronounced. However, here as well as in the joints the retrogressive changes predominated. Capillary loops and connective tissue from the marrow invaded the zones of newgrowth and helped to organize the necrotic cartilaginous masses. Thus, an irregular and variegated appearance of the epiphyseal line and the cartilage layers resulted. A fibrous vascularized tissue developed in the normally liquefied and cystic central portions of the ribs. In other places calcification and ossification of the degenerated material were seen.

Periosteum and Bone Marrow: At early stages the fibrils of the interstitial connective tissue of the bone marrow and the periosteum became swollen and torn apart, and liquid accumulated in the interstices between the fibers. At later stages, however, a shrinkage of the fibrils was noticeable; their density increased and they underwent hyalinization and sclerosis. Associated with these alterations the following changes in the various connective tissue cells were noted, especially in the marrow of the epiphysis in areas adjoining the insertion of the ligaments: (1) an edematous

swelling led to the appearance of a soft myxomatous tissue containing a limited number of star-like fibrocytes with elongated branching processes; (2) the fibrocytes became converted into precartilaginous and into typical euhyaline cartilage cells embedded in intercellular substance; and (3) a proliferation of fibrocytes resulted ultimately in the development of a dense fibrous tissue.

The first named reaction was usually seen at early stages of the injections of extract. The capillaries were enlarged and slight to moderate extravasations of blood could be detected. The edematous condition of the tissue became more and more pronounced (Fig. 6). Owing to increased pressure, a thinning and absorption of the preëxistent trabeculae and of the bony border lamella of the joints occurred.

The second type of alterations, which led to the appearance of cartilaginous islands within the marrow, was encountered at later stages. In the peripheral parts of such cartilaginous areas ordinary fibrocytes were present, which toward the center gradually assumed the shape of precartilaginous, and finally of typical cartilage cells, thus indicating a direct conversion of fibrocytes into cartilage. In the portions most centrally located, probably due to insufficient nourishment, the cartilage secondarily underwent degeneration and liquefied (Fig. 7). In other cases the cartilage cells invaded and helped to dissolve the osseous substance.

The third type of change was likewise seen after the administration of the extract over long periods of time. In this type the proliferation of fibrocytes went hand in hand with the production of dense collagenous fibers, and it was a dense fibrocytic tissue which participated in the destruction of preexisting bony material (Fig. 8). The resorption of bone as well as the retrogressive processes in the bone marrow led in some instances to the production of at first small, and later medium sized and large cysts.

As seen also in the young mature guinea pigs in localized areas, in particular in the epiphysis, a conversion of reticulum cells of the bone marrow into pseudoxanthomatous and fat cells was observed. However, in contrast to the changes seen in young animals, no appreciable growth of cartilage cells in the periosteal tissue was noticeable in this series.

Discussion

In mature guinea pigs anterior pituitary hormone exerts both growth-promoting and degenerative effects on the cartilage, as is also the case in immature guinea pigs. The proportion of these two effects, however, depends on the age of the animals and on the amount of responsive tissue present. In animals weighing 400 gm. the epiphyseal cartilage is almost completely preserved and still possesses the ability to grow under normal conditions. After administration of anterior pituitary hormone it reacts in very much the same way as the cartilage of immature guinea pigs, namely, with degeneration and proliferation. The proliferative process may be so strong that the tendency to osseous closure of the epiphyseal disc, which ordinarly becomes manifest at this age, may be overcome for a certain time. This effect is, however, not indefinitely maintained. During the 2nd month of treatment osseous closure of the epiphyseal zone sets in, either because the stimulus has become less effective or because the growth capacity of the cartilage is exhausted.

In the joints proliferation of the cartilage is quite pronounced, especially in the transitional layer, whereas degenerative processes are less accentuated. Taken together with the capillary loops advancing from the bone marrow, a condition is created which closely resembles the arthropathic lesions of the acromegalic type which we have reported previously.[7]

In guinea pigs weighing 750 to 900 gm., after treatment with anterior pituitary hormone a corresponding reaction of the epiphyseal cartilage takes place, but it is more or less rudimentary inasmuch as in these animals body growth has ceased and the structure of the epiphyseal zone, which is characteristic in young animals, has now disappeared. Therefore, the cartilage of the joint provides a more suitable test object for the present study. Some proliferation of the cartilage cells still occurs in the early stages of the experiments. However, degeneration predominates after 4 or more weeks of treatment, although considerable proliferation of the cartilage may be present even at this stage. But it is very likely that the overgrowth on the surface of the joint represents in part at least a non-specific regenerative process which is not primarily due to the action of the hormone. As to the speci-

ficity of these lesions, it must be stated that while superimposed mechanical factors and regeneration may always play a rôle, both the growth-promoting and the degeneration-producing actions of the anterior pituitary hormone create a condition highly favorable for the initiation and the further development of pathological changes. The severe arthropathic changes which were produced by the anterior pituitary hormone in old animals are comparable to those taking place in arthritis deformans in man.

The alterations in the ribs, which we observed after administration of anterior pituitary extracts, are the result of the same fundamental processes. Degeneration of cartilage, which to some extent is seen also in normal old animals, is greatly enhanced under these experimental conditions, but whereas ordinarily the degenerated areas become calcified to a large extent and only a little ossification occurs, under the influence of the extract a pronounced organization of necrotic material by connective tissue and capillaries originating in the bone marrow takes place.

According to the proportion of proliferative and degenerative changes taking place at the various ages under the influence of anterior pituitary extract, the following gradations can be made.

1. In immature guinea pigs weighing up to 200 gm., degeneration of the cartilage occurs but is transitory. Proliferation is marked and persists as long as it is not overtaken by ossification, which is also increased. Fibrous changes in the bone marrow are only slight. There exists, however, a tendency of the capillaries of the bone marrow to corrode the bony border lamella of the joint and to produce initial stages of arthopathic lesions of acromegalic type.

2. In young mature guinea pigs weighing up to 400 gm., proliferation is about as strong as in immature animals and the retrogressive changes become more accentuated, indicated especially by more extensive arthropathic changes in the joint. The capillaries of the bone marrow show increased resorptive activity and the connective tissue undergoes more fibrosis as compared with immature animals under the corresponding conditions.

3. In older mature guinea pigs weighing up to 900 gm., the retrogressive changes predominate over the proliferative changes. Diffuse degenerative alterations of the cartilage are called forth by the extract and, in cooperation with processes of organization

and regeneration, arthropathic lesions of a deforming type result. The tendency of the bone marrow to undergo fibrosis and degeneration is more pronounced than in the first two groups.

We may then conclude that the action of an anterior pituitary hormone interferes with the normal balance between cells and intercellular ground substance of the cartilage. This interference manifests itself in two different directions. If the hormone is allowed to act on young cartilage, the cells of which possess a certain potency to grow, the proliferation of these cells is increased. But if the cartilage cells have only very little growth tendency, as is the case in older animals, the growth stimulation is less prominent than the occurrence of degenerative changes. With special reference to the process of ageing of cartilaginous tissue this would mean that by the application of hormone preparations, ageing might be delayed at certain stages of development, whereas in older animals it is accelerated by these means. The histogenetic mechanism underlying these processes is complicated by the participation of the highly stimulated connective tissue in different parts of the skeleton. This actively growing tissue, which itself may undergo secondary changes, such as mucoid degeneration and cyst formation, under certain conditions causes solution processes and thus affects the structure of the cartilage of the ribs and joints, as well as of the osseous cortex and the bony trabeculae.

Summary and Conclusions

In the euhyaline cartilage of sexually mature guinea pigs anterior pituitary extract of cattle causes both proliferative and retrogressive changes similar to those found in immature guinea pigs. With increasing age of the animals the tendency of the hormone to produce proliferation of the cartilage decreases, whereas the tendency to call forth degeneration increases. The retrogressive changes increasing with advancing age consist of liquefaction and formation of cysts in the cartilage, as well as of ulcerations on free surfaces of the cartilage of the joint. Regenerative growth of the cartilage is stimulated even in old animals by injections of anterior pituitary extract. Organization of the areas of degeneration takes place by means of ingrowth of vascularized connective tissue, or by ossification, or by both of these

processes. Thus, an anterior pituitary hormone is able to produce in older guinea pigs severe arthropathic lesions, which, once they have been initiated, may be aggravated by mechanical factors in association with non-specific regenerative processes. These various factors may stimulate the growth of connective tissue, which is able to dissolve and replace preëxisting bone. In the epiphysis the rarefaction of bone, followed by retrogressive changes in the connective tissue which had invaded and replaced the bone, may give rise to the formation of cysts.

NOTE: The authors wish to express their gratitude to Dr. Leo Loeb for his advice and interest in their work.

We are indebted to Mr. S. J. Hayward for the microphotographs.

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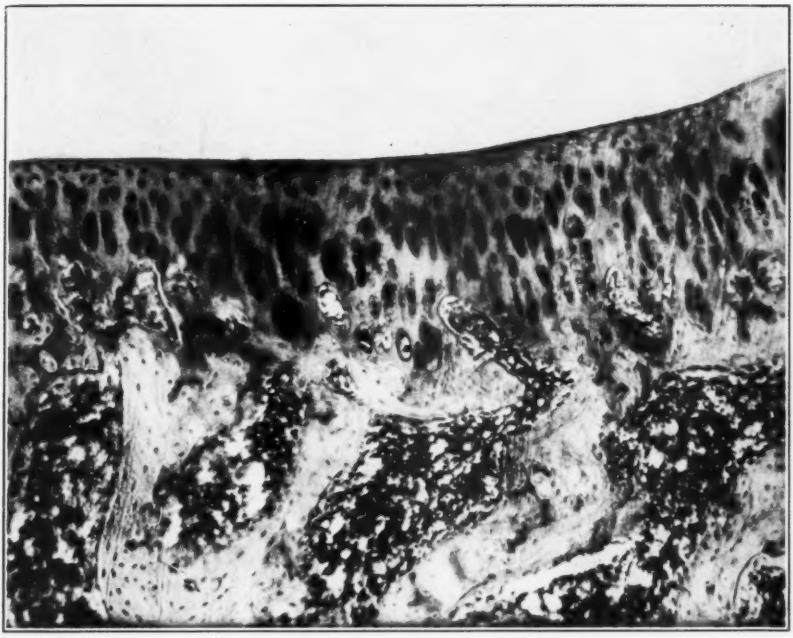
DESCRIPTION OF PLATES

PLATE 90

FIG. 1. Surface of the knee joint of a guinea pig weighing 380 gm. at the beginning of the experiment. This animal received 2 cc. of acid extract of cattle anterior pituitary gland daily for a period of 30 days. Hypertrophy and hyperplasia of the cartilaginous cell layers is present. Some degenerated cells are to be seen. The capillary loops coming from the bone marrow perforate the bony border lamella and invade the cartilage of the joint. × 120.

FIG. 2. Epiphyseal bone marrow of a guinea pig weighing 425 gm. at the beginning of the experiment. This animal had been injected with 2 cc. of cattle anterior pituitary gland extract daily for 4 days. Islands of pseudoxanthomatous cells are seen. × 220.



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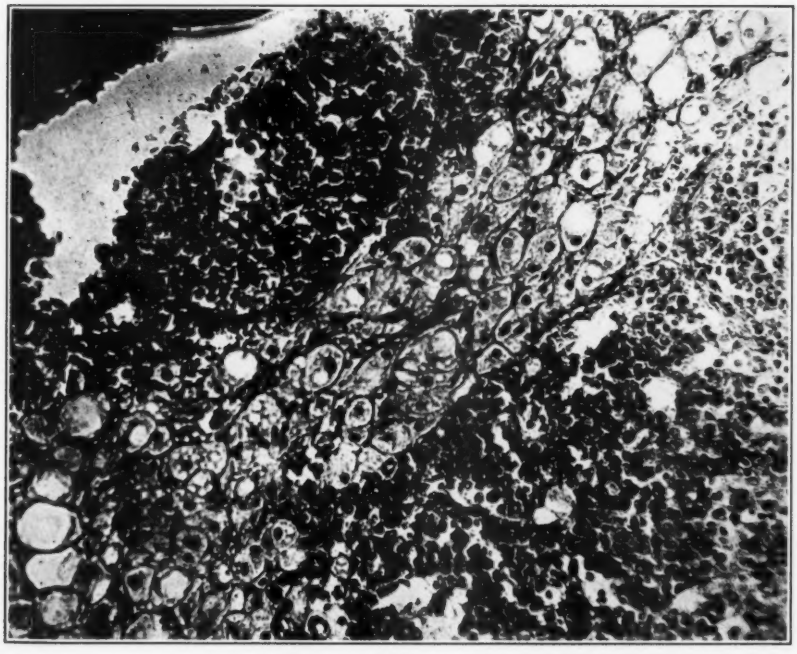

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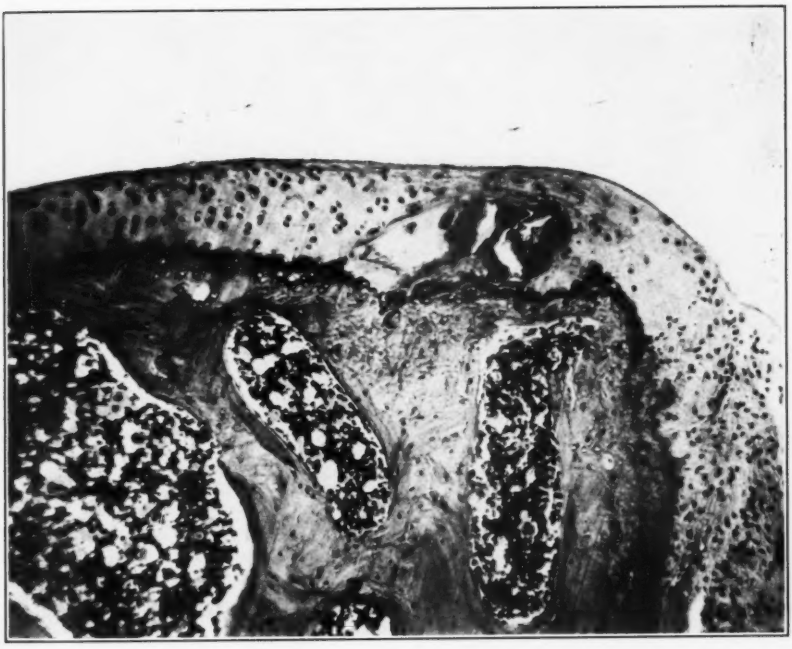
PLATE 91

FIG. 3. Surface of the head of the femur of a guinea pig weighing 750 gm. at the beginning of the experiment and which had received 4 cc. of the extract daily for 60 days. Some hyperplasia of the cartilage cells as well as localized but marked degeneration of the cartilaginous articular surface is seen. × 150.

FIG. 4. Knee joint of the same guinea pig illustrated in Fig. 3. Ulceration of the articular cartilage with partial baring of the subchondral bone is seen. The persisting cartilage shows hypertrophy as well as areas of degeneration. Capillary loops from the bone marrrow advance towards the surface of the joint. × 150.



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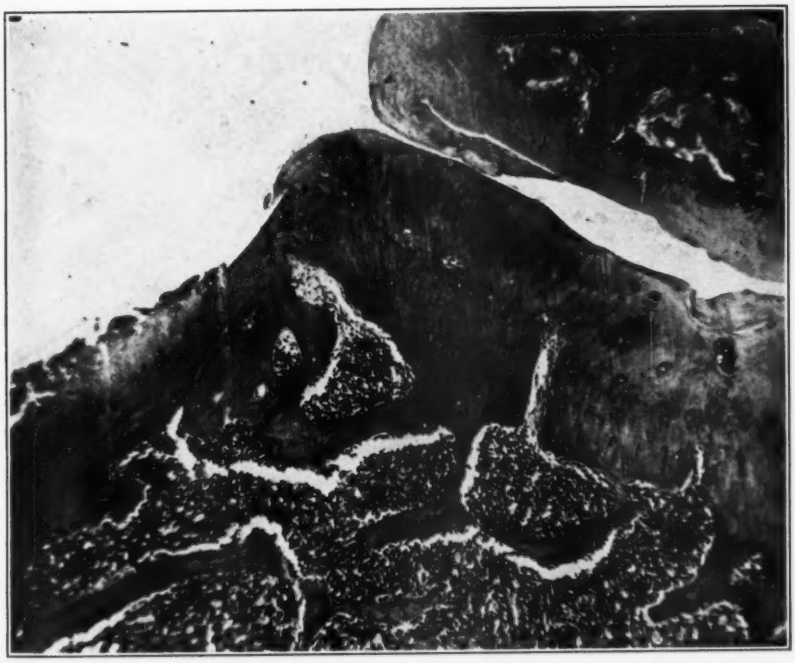
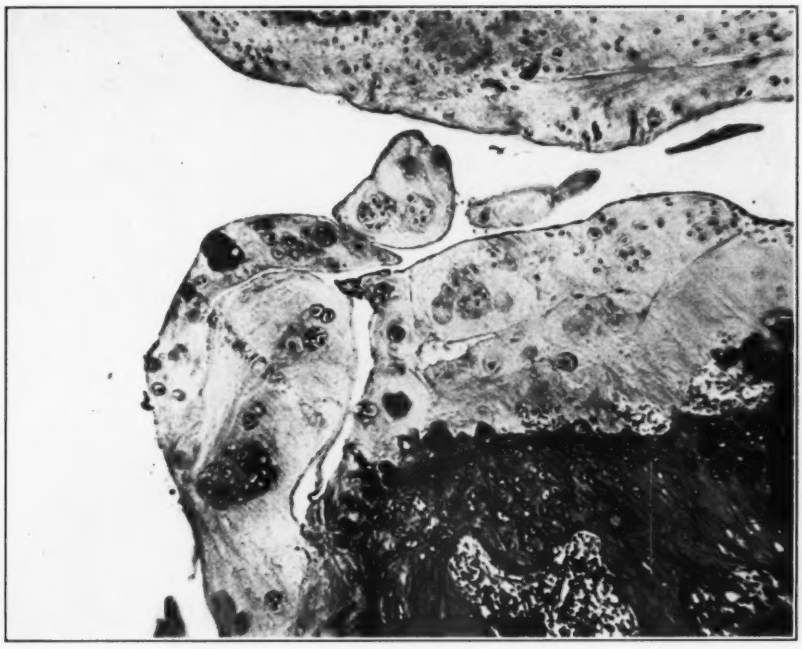


4

PLATE 92

FIG. 5. A different area from the joint shown in Fig. 4. Diffuse degeneration of hypertrophic cartilage and formation of villi on the free surface of the joint is present. × 150.

FIG. 6. Epiphyseal bone marrow of a guinea pig weighing 760 gm. at the beginning of the experiment and which had been injected with 4 cc. of the extract daily for 6 days. A formation of cysts has taken place in the myxoid connective tissue. × 150.



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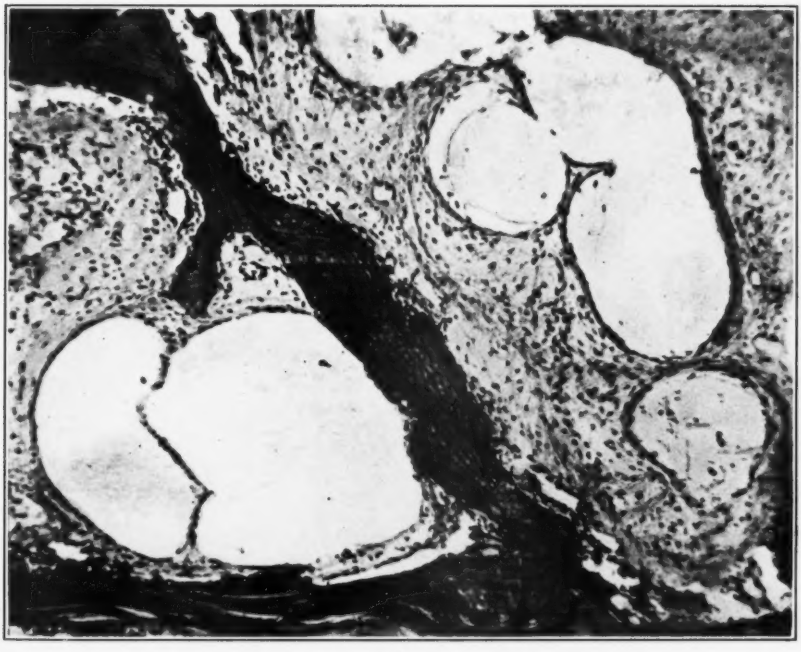

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PLATE 93

FIG. 7. Epiphyseal bone marrow of a guinea pig weighing 875 gm. at the beginning of the experiment and which had been injected with 4 cc. of the extract daily for 14 days. Formation of cartilage in the bone marrow with liquefaction in the center is to be seen. × 150.

FIG. 8. Epiphyseal bone marrow of the same guinea pig illustrated in Fig. 3. Resorption and substitution of trabeculae by fibrous tissue is present. × 220.

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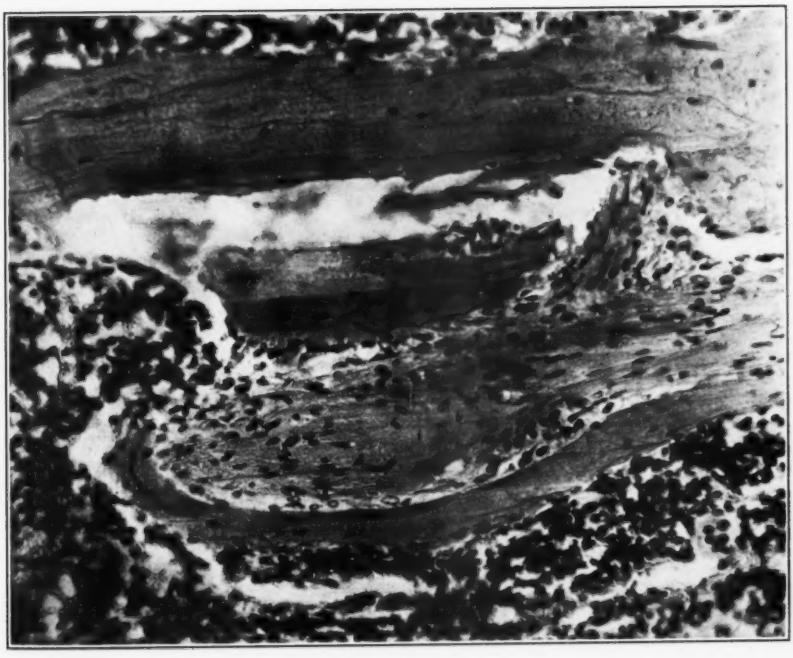

8

INTRACELLULAR BACILLI IN INTESTINAL AND MESENTERIC LESIONS OF TYPHOID FEVER *

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In 1937 Goodpasture [1] reported the occurrence of small Gram-negative intracellular bacilli, judged to be *Eberthella typhi*, within the cytoplasm of young plasma cells located in the lymphoid follicles of the ileum and in mesenteric lesions of early cases of typhoid fever. Larger Gram-negative bacilli were found concurrently in these lesions in necrotic macrophages. These observations were made on 5 cases of human typhoid fever which had come to autopsy early in the disease. It was concluded that *E. typhi* is capable of growing in both of these situations, and the inference was made that the young plasma cell is an essential cellular host in the typical human disease and serves as a nourishing and protecting medium, not only during the period of incubation but throughout the active course of typhoid fever. He was led to study the lesions of typhoid fever after the observation of small, intracytoplasmic colonies of bacilli in the entodermal epithelial cells of the chorio-allantois of chick embryos infected with *E. typhi*.[2]

It is the purpose of this paper to report a similar study of 6 additional cases of typhoid fever.†

Zenker-fixed paraffin sections stained in Wright's stain (60 drops to 100 cc. of distilled water) for about 4 hours, differentiated in absolute ethyl alcohol, cleared in xylol and mounted in cedar oil were used for study. With this method the intracytoplasmic bacillary forms are somewhat inconspicuous owing to the blue-staining properties of both the cytoplasm of the plasma cells and the bacteria, in conjunction with the relatively small size of the bacterial forms. The following staining method was therefore

* Received for publication June 5, 1939.

† Of the cases studied, 2 came from the Department of Pathology of Vanderbilt University. Material from 1 case was supplied by Dr. W. A. DeMonbreun from the Pathological Laboratory of the Nashville General Hospital. Sections from 3 cases were kindly sent by Dr. W. D. Forbus from the Pathology Department of Duke University Hospital.

devised whereby better contrast could be obtained between the bacteria and the cytoplasm of the cells.

1. Stain in Weigert's iron hematoxylin solution for 1 minute or less.
2. Wash in 50 per cent alcohol acidulated to 0.1 per cent hydrochloric acid.
3. Stain in Goodpasture's carbol-aniline-fuchsin solution for 1 minute.
4. Decolorize in a 5 per cent aqueous solution of acetic acid until the red color disappears or the sections remain a light pink.
5. Repeat steps 3 and 4 three times.
6. Wash in water.
7. Stain in a 0.01 per cent light green solution, acidulated to 0.2 per cent acetic acid, for about 1 minute or until the section has a light green hue. Overstaining is to be avoided.
8. Wash in water, differentiate in 95 per cent alcohol, dehydrate in absolute alcohol, clear in xylol and mount in balsam.

The nuclei are stained black, the nucleoli red, the cytoplasm a pale green, bacteria a brilliant red, red blood cells red and other structures green.

Abstract of Cases

Case 1, V–38–111: A 56 year old negro was admitted to the Vanderbilt Hospital in a stuporous condition with a story of diarrhea and fever of 4 days duration. The blood culture was positive for *E. typhi*. The course of illness was one of profound toxemia and was steadily downhill. Death occurred on the 3rd hospital day.

Autopsy showed hyperplastic Peyer's patches and solitary follicles in the distal 36 inches of the ileum. The lesions were in an early ulcerative phase. There was a mesenteric lymphadenitis and focal necrosis of the liver, spleen and kidneys. The blood culture and culture of the intestinal lesions were positive for *E. typhi*.

Intracytoplasmic bacillary forms were demonstrated in the intestinal lesions without difficulty. Cells of this type were rarely found in sections of the mesenteric nodes.

CASE 2, G–38–127: A 22 year old negro was admitted to the Nashville General Hospital 13 days before death with a history of fever of 4 days duration. The Widal test showed agglutination at 1:640 and the stool culture yielded *E. typhi.*

Autopsy revealed classical, hyperplastic lymphoid lesions in the distal 12 inches of the ileum and the proximal 24 inches of the colon. The appendix was inflamed and the mesenteric nodes were swollen. Microscopic studies revealed classical, pre-ulcerative, early necrotic, typhoid intestinal lesions, mesenteric lymphadenitis, and focal areas of necrosis in the spleen, liver, pancreas and adrenal. A blood culture at autopsy yielded a pure culture of *E. typhi.*

Intracytoplasmic bacillary forms were relatively numerous in the plasma cells in the involved Peyer's patches. They were demonstrated in the colon and in the mesenteric nodes. None was found in the appendix.

CASE 3, Duke No. 173: A 20 year old negro was admitted to the Duke University Hospital 5 days before death with a history of headache for 15 days and fever for 8 days. The blood culture was positive for *E. typhi.*

Autopsy revealed early ulceration of Peyer's patches and hyperplastic follicles in the proximal colon. There was a mesenteric lymphadenitis, an inflamed appendix and focal areas of necrosis in the spleen and liver.

Intracytoplasmic bacillary forms were observed in plasma cells in sections of the involved areas of the ileum and colon. The mesenteric nodes were not studied and no bacterial forms were found in the appendix.

CASE 4, Duke No. 257: A 15 months old white boy was admitted to the Duke University Hospital 9 days before death with a history of fever of 2 weeks duration. The blood culture was positive for *E. typhi* on admission and at autopsy.

Ulcerative lesions were present, but not marked, in the Peyer's patches and in the colon. Microscopically the lesions were in the ulcerative stage. The cellular response was atypical in that there were many more large mononuclear cells and fewer plasma cells than usual. There was a marked mesenteric lymphadenitis.

Intracytoplasmic bacillary forms were demonstrated infrequently in occasional plasma cells in a section of a Peyer's patch. None was found in other sections.

CASE 5, Duke No. 298: An 8 year old negro boy ill for 2 weeks before admission to the Duke University Hospital died on his 2nd hospital day.

At autopsy ulcerated lesions were present in the cecum, ascending colon and Peyer's patches. Microscopically the hyperplastic and ulcerative lesions in the intestines showed a somewhat atypical cellular response. In some areas there were large collections of polymorphonuclear leukocytes. There was a marked mesenteric lymphadenitis.

Plasma cells in a section of a Peyer's patch showed infrequent intracytoplasmic bacillary forms. None was found in the colon. Typical intracytoplasmic colonies were observed in large plasma cells in a section of a mesenteric node.

A 6th case, in which no intracytoplasmic bacillary forms were found, may be compared with the above group.

CASE 6, V–37–144: A 15 year old girl was admitted to the Vanderbilt Hospital in a semistuporous condition with a history of fever of 2 weeks duration. On her 4th hospital day a perforation was suspected and an exploratory operation was performed, during which two perforated ulcers in the lower ileum were closed. The course of illness was downhill until she died on the 7th hospital day.

Autopsy showed a diffuse peritonitis, deeply ulcerated Peyer's patches in the ileum and ulcerated lesions in the cecum and ascending colon. *E. typhi* was isolated from the bile but was not recovered from cultures of the blood stream or from the ulcers. Microscopically the lesions were necrotic and deeply ulcerated.

DISCUSSION

This study confirms the previous observations of Goodpasture that the intracytoplasmic bacillary forms within plasma cells of intestinal and mesenteric lymphoid lesions of typhoid fever are most numerous in early cases. They are found in areas where necrosis has not yet taken place or is limited in extent and where cellular hyperplasia and infiltration are marked. They are most frequently found in hyperplastic areas over which the mucosa is still intact.

The observations here recorded supplement the previous report in that intracytoplasmic forms were demonstrated in colonic lesions as well as in lesions in the ileum and in involved mesenteric nodes. They were also demonstrated in childhood lesions of

typhoid where the cellular reaction consists more of a large mononuclear infiltration with an associated polymorphonuclear invasion.

In one instance also the small bacillary forms were found within the cytoplasm of a lymphoblast or plasma cell in the process of mitosis. This observation indicates that the bacilli may cause little or no injury to the host cell, and that the latter, stimulated to mitosis, might give rise to two infected cells without reinfection. The frequent observation of two or more infected plasma cells in close proximity to each other suggests the possibility also of reproduction of infected cells.

The intracytoplasmic colonies of bacillary forms, as seen in plasma cells, are surrounded by a narrow clear zone. In some cells there is only a single colony composed of a few bacteria while in others there are as many as six colonies. Some colonies contain a large number of bacteria, perhaps 100 or more. In occasional cells containing large colonies the vacuoles in which they lie appear to have ruptured and the bacilli appear to be exuding into the adjacent tissue. The bacillary forms are Gram-negative and all indications are that they are small forms of *E. typhi.*

The apparent regularity in the occurrence of intracellular bacterial forms within young plasma cells in the intestinal and mesenteric lesions in early cases of typhoid fever indicates that they are an essential component of these classical early lesions and that they are of importance in the pathogenesis of this disease.

Summary and Conclusion

1. Gram-negative, intracytoplasmic bacillary forms, judged to be *E. typhi,* have been found in the cytoplasm of young plasma cells located in the lymphoid follicles of the ileum, colon and mesenteric lymph nodes in 5 cases of early typhoid fever.

2. A method for staining intracellular bacteria is described.

3. It is concluded that the presence of these bacillary forms within the plasma cell is an essential part of the early, classical, intestinal and mesenteric lesions of typhoid fever.

REFERENCES

1. Goodpasture, E. W. Concerning the pathogenesis of typhoid fever. *Am. J. Path.*, 1937, 13, 175–185.

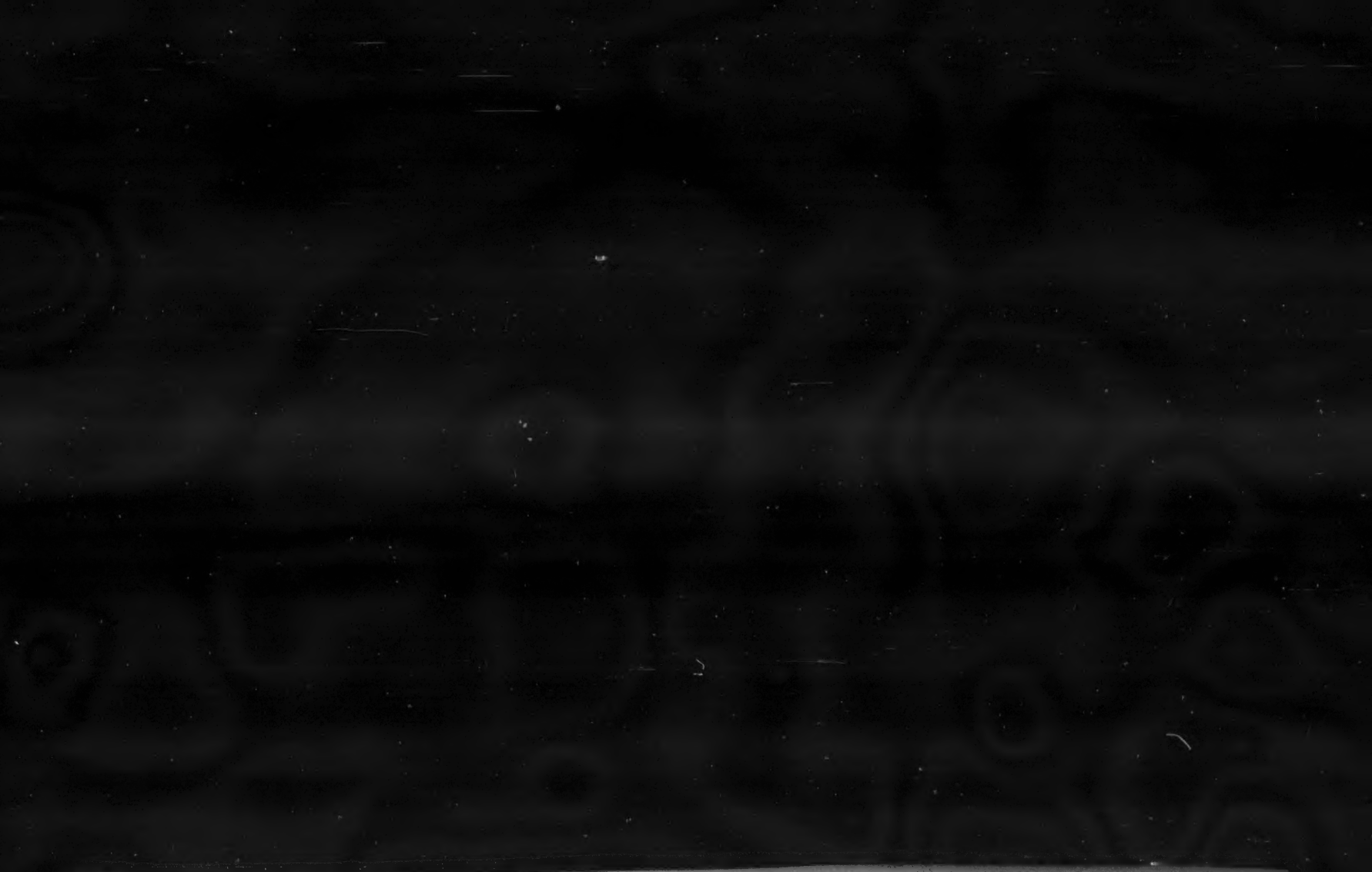
2. Goodpasture, E. W., and Anderson, K. The problem of infection as presented by bacterial invasion of the chorio-allantoic membrane of chick embryos. *Am. J. Path.*, 1937, 13, 149–174.

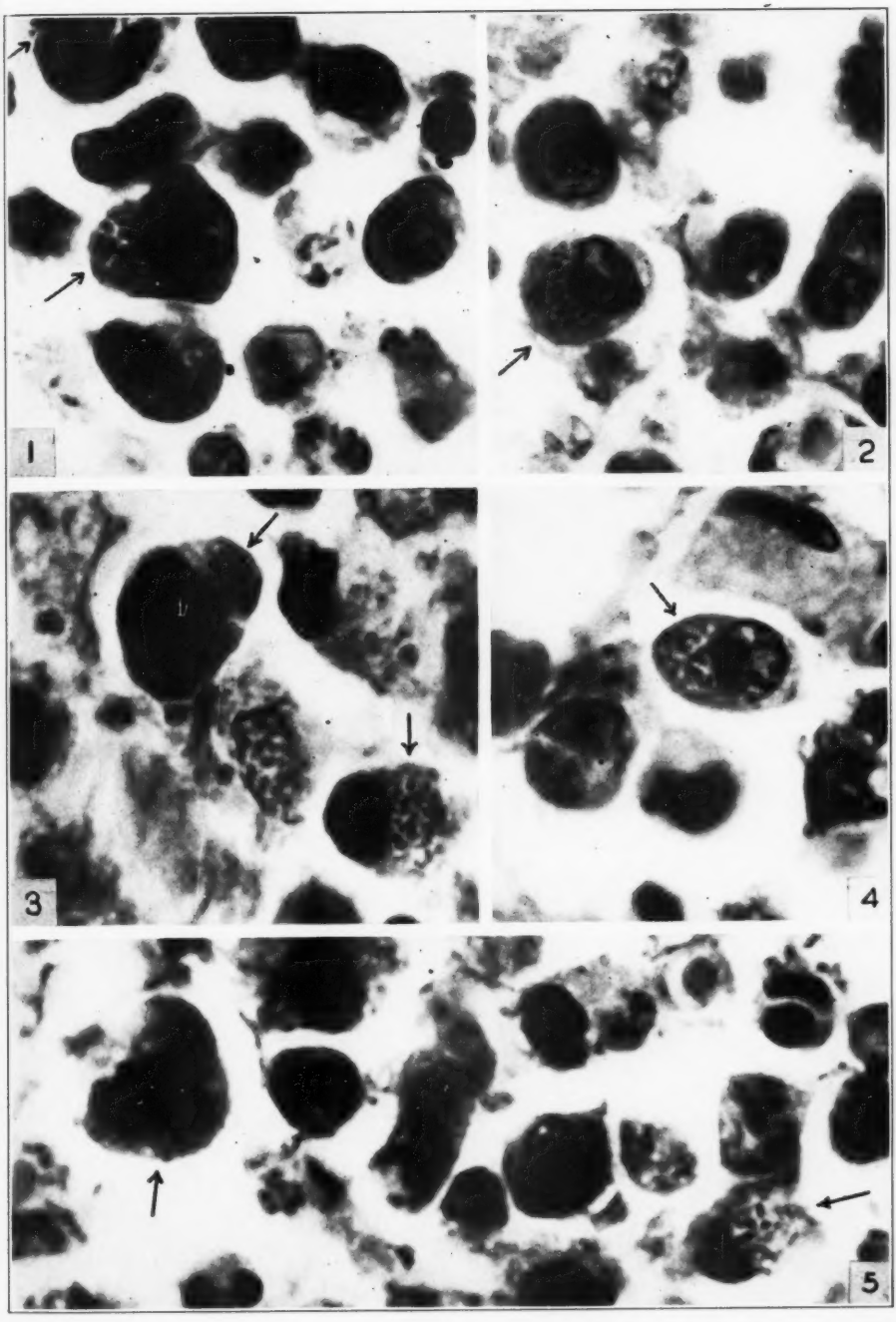
DESCRIPTION OF PLATE

PLATE 94

FIGS. 1–4. Microphotographs showing young plasma cells of a persisting follicle in a Peyer's patch. The arrows point to groups of intracellular bacilli. Note the encapsulating material about the bacilli. Basic fuchsin-light green stain. × 2500.

FIG. 5. The cell on the left containing intracellular bacilli is undergoing mitosis. × 2500.





Adams

Intracellular Bacilli in Typhoid Fever

CARCINOMA OF THE LUNG *

An Analysis of Seventy-Four Autopsies

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Two previous communications from this laboratory have dealt respectively with 40 cases of primary carcinoma of the biliary system [1] and 40 cases of primary carcinoma of the pancreas.[2] The present study deals with 74 cases of primary carcinoma of the lung. As in the previous communications, this report is principally concerned with the site and structure of the primary growth and with its spread locally and to distant parts. The clinical manifestations, their duration, and the immediate causes of death are also briefly considered.

Race, Sex and Age: The 74 cases of carcinoma of the lung were encountered in 6623 autopsies on individuals over 1 year of age performed between Jan. 1, 1931, and June 30, 1938 by the staff of the Department of Pathology of the Charity Hospital of Louisiana at New Orleans. Forty-seven patients were white (42 male, 5 female) and 27 negro (26 male and 1 female). The youngest patient was 21 and the oldest 75 years of age. One died in the 3rd, 4 in the 4th, 13 in the 5th, 33 in the 6th, 19 in the 7th, and 4 in the 8th decade of life (Tables I, II and III).

Site and Structure of Neoplasms: The main growth was located in the right lung in 38 cases and in the left lung in 33. In the 3 remaining cases the site could not be determined. In 35 cases the primary growth was located in one bronchus or the other, 6 in this group being located near or at the bifurcation of the trachea (Fig. 1). Twenty-eight growths were located in a branch of a bronchus (Fig. 2).

The diameter of the mass forming the primary growth varied from 2 to 15 cm. Ulceration of the bronchial mucosa was frequently observed, together with involvement of the bronchial wall and of the underlying pulmonic tissue. The affected bronchus was usually identified near the periphery of the growth and not

* Received for publication March 11, 1939.

TABLE I

Data on Patients with Squamous Cell Carcinoma

Number of case	Age	Sex and race	Site *		Spread		Clinical manifestations	Duration of illness	Cause of death
			Right	Left	Regional	Distant			
	yrs.							*mos.*	
S–1 33–1362	33	M W	B		Lymph nodes	Lymph nodes, pancreas	Pain, cough, dyspnea	3	Carcinoma
S–2 35–806	42	M W		B	Lymph nodes		Cough	6	Carcinoma
S–3 37–1021	42	M C		B U	Ribs	Lymph nodes	Pain, dyspnea, hemoptysis	9	Carcinoma
S–4 32–540	49	M C	B U		Lymph nodes	Lymph nodes	Cough, dysphagia	1	Carcinoma
S–5 32–277	50	M W		B U	Lymph nodes	Lymph nodes	Pain, cough, loss of weight	5	Abscess of lung
S–6 32–1047	50	M C	Lung	Lung	Lymph nodes, pericardium	Liver, adrenals, skeleton	Pain, loss of weight	2	Carcinoma
S–7 38–233	50	M C		B	Lymph nodes		Hemoptysis	1	Carcinoma
S–8 31–371	51	M W		B U	Lymph nodes	Lymph nodes			Abscess of lung
S–9 34–309	51	M C	B		Lymph nodes	Liver, adrenals, pancreas	Pain, cough, dyspnea, hemoptysis	3	Carcinoma
S–10 36–1390	51	M W		Lung	Pleura		Pain	3	Carcinoma
S–11 33–448	53	M W		B U	Lymph nodes		Pain, dyspnea	2	Carcinoma
S–12 34–915	53	M C	B L		Lymph nodes	Liver, pancreas	Pain	9	Abscess of lung
S–13 33–83	54	M W	B				Cough, dyspnea, hemoptysis	6	Abscess of lung
S–14 33–275	54	M W	B L				Cough	5	Abscess of lung
S–15 37–526	54	M C	Lung		Pleura		Cough, loss of weight	5	Carcinoma
S–16 36–420	55	M W		B	Pleura	Kidney	Pain, cough, dyspnea	5	Carcinoma
S–17 38–176	55	M C	B L			Lymph nodes	Dyspnea, dysphagia	4	Carcinoma

* B = bronchus; U = upper; L = lower; M = middle.

S–18 37–1037	57	M W		B	Lymph nodes, pleura	Liver, skeleton, skin	Pain, cough, loss of weight	1	Carcinoma
S–19 33–563	58	M W	B				Cough		Carcinoma
S–20 37–885	58	M C	B L		Lymph nodes		Dyspnea	1	Obstruction vena cava inferior
S–21 38–111	58	M C	B		Lymph nodes		Pain, cough, hemoptysis	1	Carcinoma
S–22 32–124	59	M W		B L	Lymph nodes, pleura		Pain, cough, dyspnea	18	Abscess of lung
S–23 33–1277	59	M C	B U			Intestine		12	Abscess of lung
S–24 38–114	59	M C		B	Lymph nodes, pericardium	Liver	Cough, loss of weight, hemoptysis	5	Carcinoma
S–25 36–1512	60	M W		B	Lymph nodes	Skeleton	Cough, loss of weight, hemoptysis	6	Carcinoma
S–26 32–967	61	M W		B L	Lymph nodes, pericardium		Pain, dysphagia	5	Carcinoma
S–27 36–1083	61	M W	Lung		Lymph nodes		Pain	4	Carcinoma
S–28 37–333	62	M W		B	Lymph nodes, pleura, pericardium		Pain, cough, loss of weight, hemoptysis	12	Abscess of lung
S–29 38–55	63	M W	Lung			Liver	Pain, dyspnea, loss of weight	7	Carcinoma
S–30 37–840	63	F W		B U	Lymph nodes, pericardium	Kidneys	Pain	5	Carcinoma
S–31 35–1162	64	M W	B U		Pleura		Pain, cough, loss of weight	4	Carcinoma
S–32 37–1237	64	M C	B		Lymph nodes	Lymph nodes	Pain, dyspnea	1	Carcinoma
S–33 38–376	64	M W		B	Lymph nodes	Liver, kidneys	Dyspnea, hemoptysis	7	Carcinoma
S–34 38–77	65	M W		B			Loss of weight	3	Carcinoma
S–35–36–342	68	M C		B			Dyspnea, loss of weight	6	Carcinoma
S–36 33–1082	73	M C		B			Cough		Abscess of lung
S–37 37–1031	74	F W	Lung	Lung					Thrombosis, coronary artery

TABLE II

Data on Patients with Reserve Cell Carcinoma

Number of case	Age	Sex and race	Site *		Spread		Clinical manifestations	Duration of illness	Cause of death
			Right	Left	Regional	Distant			
	yrs.							*mos.*	
R–1 33–59	21	M C		B U	Lymph nodes, pleura	Skeleton	Paralysis	1	Carcinoma, transverse myelitis
R–2 37–54	36	M W	Lung		Lymph nodes	Kidneys	Pain, dyspnea	4	Carcinoma
R–3 37–955	40	M C		B	Lymph nodes		Pain, cough, loss of weight	1	Pneumonia, diffuse
R–4 32–354	46	M W		B U			Pain	4	Abscess of lung
R–5 36–304	47	M C	B		Lymph nodes	Liver	Cough	4	Abscess of lung
R–6 32–112	48	M W		B	Lymph nodes	Kidneys, skeleton, meninges	Dyspnea, loss of weight	3	Carcinoma
R–7 33–2	51	F C		Lung	Pleura	Liver	Pain, cough	1	Carcinoma
R–8 33–306	51	M C	Lung	Lung	Lymph nodes		Loss of weight, dysphagia	6	Carcinoma
R–9 37–1043	54	M W		B	Lymph nodes		Dyspnea		Lobectomy
R–10 35–180	56	M W	B L		Pleura	Lymph nodes, liver, pancreas	Pain	1	Carcinoma
R–11 31–99	57	M W	B U		Lymph nodes	Liver	Pain, cough		Carcinoma
R–12 36–212	57	M W		B			Cough, loss of weight, hemoptysis	3	Pneumonia, diffuse

* B = bronchus; U = upper; L = lower; M = middle.

R–13 34–785	58	M W	B				Pain, cough	2	Abscess of lung
R–14 37–790	58	M W	B		Lymph nodes		Cough, dyspnea	6	Constriction vena cava superior
R–15 36–204	59	M W	B M		Lymph nodes	Liver, adrenals, pancreas	Pain, hemoptysis	7	Carcinoma
R–16 35–1062	60	M C	B		Lymph nodes, pleura	Liver			Carcinoma
R–17 34–907	61	M W		Lung	Lymph nodes	Liver, pancreas	Pain	1	Carcinoma
R–18 34–933	68	MC		B	Lymph nodes	Lymph nodes, liver	Dysphagia	3	Carcinoma
R–19 36–490	68	M W	B L		Lymph nodes				Abscess of lung
R–20 34–905	72	M W	B L		Lymph nodes, pleura	Liver	Pain, cough, loss of weight	6	Carcinoma
R–21 36–1458	75	M W		B	Lymph nodes		Pain, cough	24	Carcinoma

TABLE III

Data on Patients with Columnar Cell Carcinoma

Number of case	Age	Sex and race	Site *		Spread		Clinical manifestations	Duration of illness	Cause of death
			Right	Left	Regional	Distant			
	yrs.							*mos.*	
C–1 31–85	39	F W	B U		Lymph nodes	Lymph nodes	Pain	1	Constriction vena cava superior
C–2 34–163	48	M C		Lung	Lymph nodes, diaphragm	Lymph nodes, liver, pancreas	Pain, dyspnea	3	Carcinoma
C–3 34–747	48	M W	B		Lymph nodes	Lymph nodes	Pain, loss of weight, hemoptysis	3	Abscess of lung
C–4 36–346	49	M W	B		Lymph nodes		Cough, hemoptysis	7	Carcinoma
C–5 35–195	50	M W	B		Lymph nodes		Pain, cough	8	Abscess of lung
C–6 36–96	52	M W	B U		Lymph nodes		Pain, dyspnea	9	Carcinoma
C–7 37–439	52	M C	B		Lymph nodes, diaphragm		Pain, dyspnea	4	Lobectomy
C–8 38–434	52	M C	B L		Lymph nodes, pleura	Liver, adrenals, pancreas			Carcinoma
C–9 34–597	54	F W	B		Lymph nodes, pleura	Liver, adrenals, pancreas, spleen	Pain, dyspnea, hemoptysis	6	Carcinoma
C–10 35–1060	54	M W	B U		Lymph nodes		Cough, dysphagia	12	Carcinoma
C–11 36–160	61	M W		B U	Lymph nodes, ribs	Lymph nodes, adrenal	Pain, cough	5	Carcinoma
C–12 38–222	62	M C	B		Lymph nodes	Adrenals, skeleton		1	Carcinoma
C–13 34–1295	64	M C	B		Diaphragm	Lymph nodes, skeleton	Cough	5	Carcinoma
C–14 35–442	65	M W		B L	Pleura, ribs	Adrenals, lymph nodes	Pain, cough, loss of weight	3	Carcinoma
C–15 34–952	67	F W	B L		Lymph nodes, pleura	Kidneys	Cough, dyspnea, loss of weight	5	Carcinoma
C–16 32–859	68	M W		B	Lymph nodes		Cough, hemoptysis	5	Carcinoma

* B = bronchus; U = upper; L = lower; M = middle.

in the center (Fig. 3). The primary growths and their metastatic foci varied in gross appearance, the variations seeming to depend principally on the rate of growth, the amount and character of the stroma, and such secondary changes as hemorrhage and necrosis, rather than upon the actual cellular structure of the tumor parenchyma. It was therefore impossible to set up definite criteria by which on gross examination the microscopic structure of the carcinoma could be predicted with any degree of certainty.

Following the histogenetic classification previously outlined by one of us (B. H.[3]), the neoplasms were divided into three groups on the basis of their microscopic structure — squamous cell, reserve cell and columnar cell carcinoma. Thirty-seven of the 74 neoplasms were squamous cell, 21 reserve cell and 16 columnar cell carcinoma.

The squamous cell carcinoma was usually composed of nests or sheets of tumor cells arranged more or less concentrically to form epithelial pearls (Fig. 4). In some growths the cells toward the center of the cell sheets disclosed varying degrees of keratinization (Fig. 5), or were transformed into scales or into cell débris (Fig. 6).

The reserve cell carcinoma was composed of sheets or solid masses of tumor cells, which formed no particular structure (Fig. 7). Usually the cytoplasm was scant and the cell borders hardly discernible. The nuclei of the cells were fairly uniform, ovate or elongated, and stained deeply. In some growths the cells seemed to be arranged in whorls (Fig. 8), in others there was a palisade arrangement of the peripheral cells (Fig. 9).

The columnar cell carcinoma was usually composed of columnar or cuboidal cells, and of solid masses of undifferentiated tumor cells. The columnar or cuboidal cells formed acinar or tubular structures which simulated in a haphazard way the normal epithelial structures of the air passages (Figs. 10 and 11). In some growths these cells were mounted on connective tissue stalks in a papillary arrangement (Fig. 12). Columnar cells forming acinar or tubular structures were occasionally observed in predominantly squamous cell growths.

In all three types of carcinoma of the lung there was a wide variation in the number of nuclei in mitosis. The amount and

B = bronchus; U = upper; L = lower; M = middle.

density of the stroma, the degree of infiltration with lymphocytes and plasma cells, and the extent of areas of necrosis and hemorrhage also varied in the individual growths, as well as in different fields of the same growth.

Manner of Spread: Local extension with involvement of the regional lymph nodes occurred in 65 of the 74 cases (87.8 per cent), and extensive distant metastases in 41 (55.4 per cent). Metastatic foci were encountered in the liver 19 times, in the pancreas and suprarenal glands 8 times each, and in the kidneys and in the skeleton 7 times each.

Clinical Course: Thirty-seven of the 74 patients complained of pain in the chest, neck or epigastrium. Thirty-five complained of cough, with or without expectoration, 21 of dyspnea, 19 of loss of weight, 15 of hemoptysis, and 6 of dysphagia.

In the 65 cases in which information was available, the illness had lasted from 1 to 24 months, with an average duration of 5 months. The newgrowth was the principal lesion and the immediate or contributory cause of death in 71 patients.

Comment

The increasing importance of carcinoma of the lung as a clinical problem is made clear in such recent publications as those of Hruby and Sweany,[4] Tuttle and Womack,[5] Rabin and Neuhof,[6] Jackson and Konzelmann,[7] Graham,[8] Kennaway and Kennaway,[9] Husted and Biilmann,[10] Simons,[11] Mattick and Burke,[12] Edwards,[13] Klotz,[14] Matz,[15] Howes and Schenck,[16] Hochberg and Lederer,[17] Tod,[18] and Ochsner and DeBakey.[19] New opportunities for the analysis and clarification of some of its morphological problems are presented in the large series of cases studied at autopsy and reported in such recent communications as those of Geschickter and Denison,[20] Neely,[21] Olson,[22] Jaffé,[23] Lindberg,[24] Samson,[25] Rice,[26] Frissell and Knox,[27] Brines and Kenning,[28] Bauer,[29] and Koletsky.[30]

There is an apparently wide variance in the conceptions of individual authors as to the histogenesis and structure of carcinoma of the lung. The available data have contributed materially to our knowledge, but no uniformity has as yet been attained in classifying these growths. The difficulty may be superficial, however, rather than essential. In our opinion a classification on a

histogenetic basis, combined with a nomenclature derived from the cell making up the growth, rather than from the structure which the cell forms, will go far toward simplification of the problem.

All carcinomas primary in the lung, it now seems clear, can be classified into one of three groups — squamous cell, reserve cell and columnar cell. The occasional overlapping of groups is not unexpected, since all carcinomas of the lung, we believe, are derived from a common ancestor cell, the reserve cell (Fried [31]).

Summary

1. Seventy-four cases of primary carcinoma of the lung were encountered in 6623 autopsies on individuals over 1 year of age. Males and females were represented in the proportion 11:1. The age range was from 21 to 75 years. The average duration of illness was 5 months. Thirteen patients died in the 5th, 33 in the 6th, and 19 in the 7th decade of life.

2. In almost half of the cases the primary growth was located in one bronchus or the other.

3. Thirty-seven of the 74 cases were squamous cell, 21 were reserve cell, and 16 were columnar cell carcinoma.

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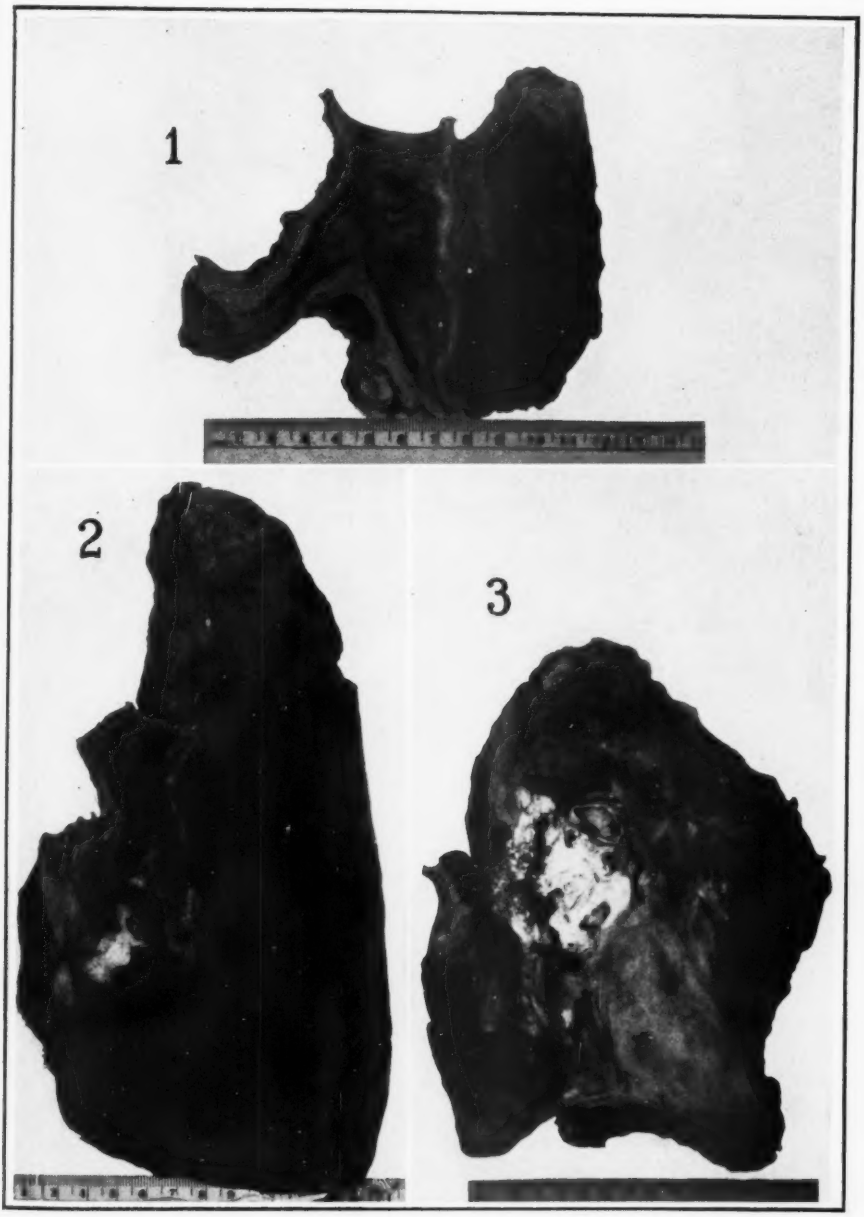
DESCRIPTION OF PLATES

PLATE 95

FIG. 1. Primary growth in the right bronchus at the bifurcation of the trachea. The neoplastic infiltration extends through the entire thickness of the wall (R 13).

FIG. 2. Primary growth in the hilus of the right lung in the wall of the bronchial branch to the lower lobe (R 20).

FIG. 3. Primary growth in the hilus of the left lung, arising from the bronchial wall. The bronchus lies along the anterior margin of the growth and not in the center (S 34).



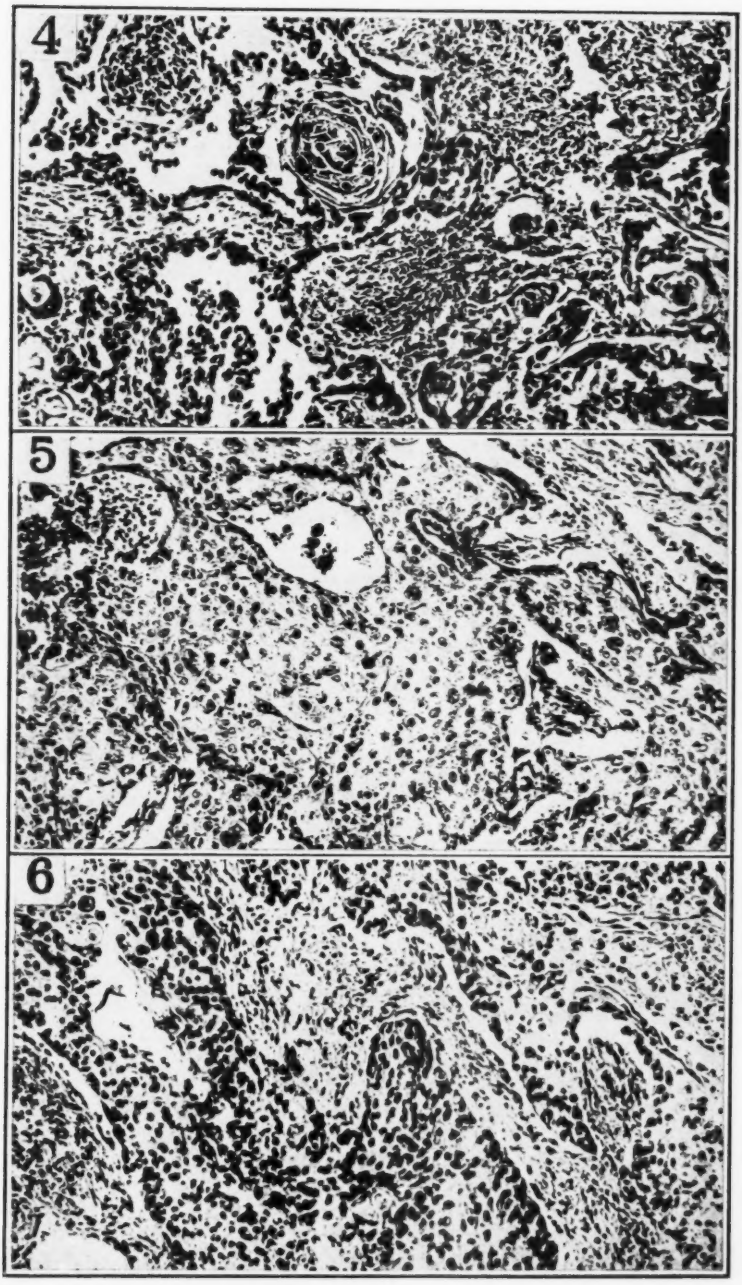
D'Aunoy, Pearson and Halpert Carcinoma of Lung

PLATE 96

FIG. 4. Squamous cell carcinoma composed of nests or sheets of tumor cells arranged more or less concentrically to form epithelial pearls (S 14).

FIG. 5. Squamous cell carcinoma. The cells toward the center of the cell sheets disclose varying degrees of keratinization (S 24).

FIG. 6. Squamous cell carcinoma. The cells toward the center of the cell sheets disclose varying degrees of keratinization and a cell débris (S 13).



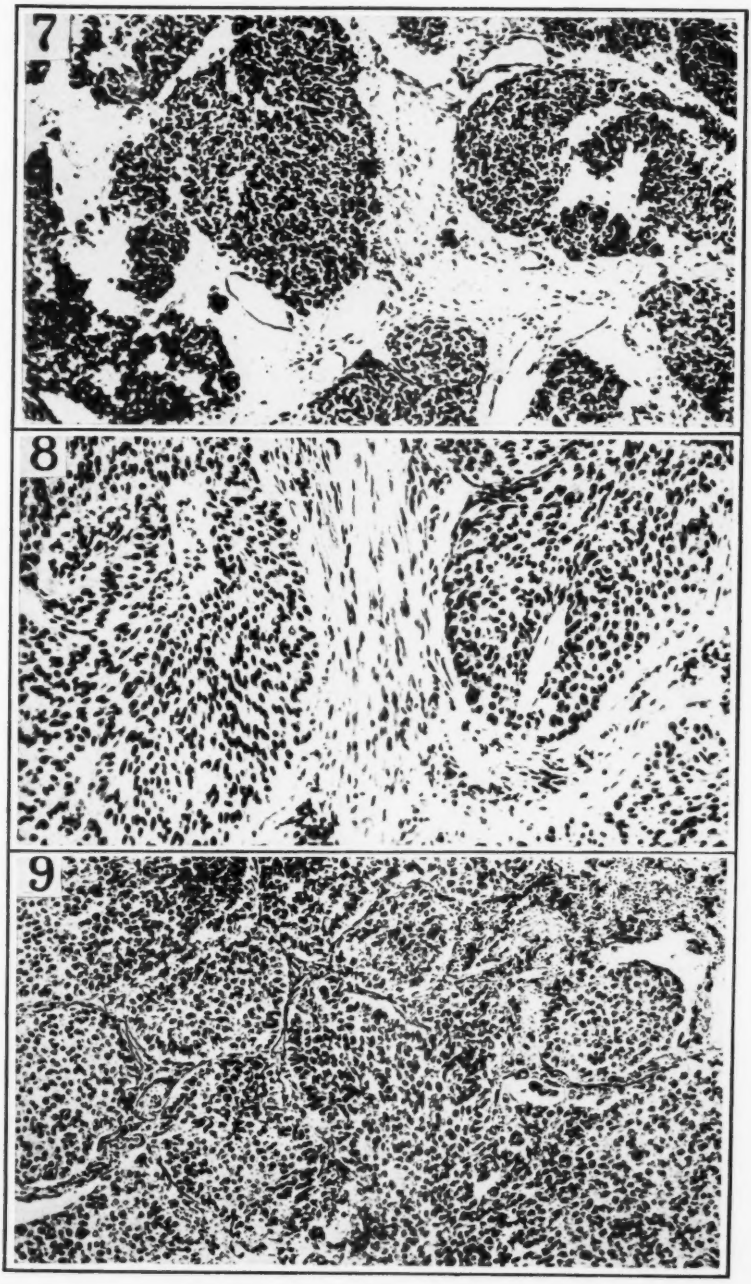
D'Aunoy, Pearson and Halpert Carcinoma of Lung

PLATE 97

FIG. 7. Reserve cell carcinoma composed of sheets or solid masses of tumor cells forming no particular structure. The cytoplasm is scant and the cell borders hardly discernible. The cell nuclei are fairly uniform, ovate or elongated, and stain deeply (R 6).

FIG. 8. Reserve cell carcinoma. The cells seem to have a whorl-like arrangement (R 19).

FIG. 9. Reserve cell carcinoma. A palisade arrangement of the peripheral cells is seen (R 20).

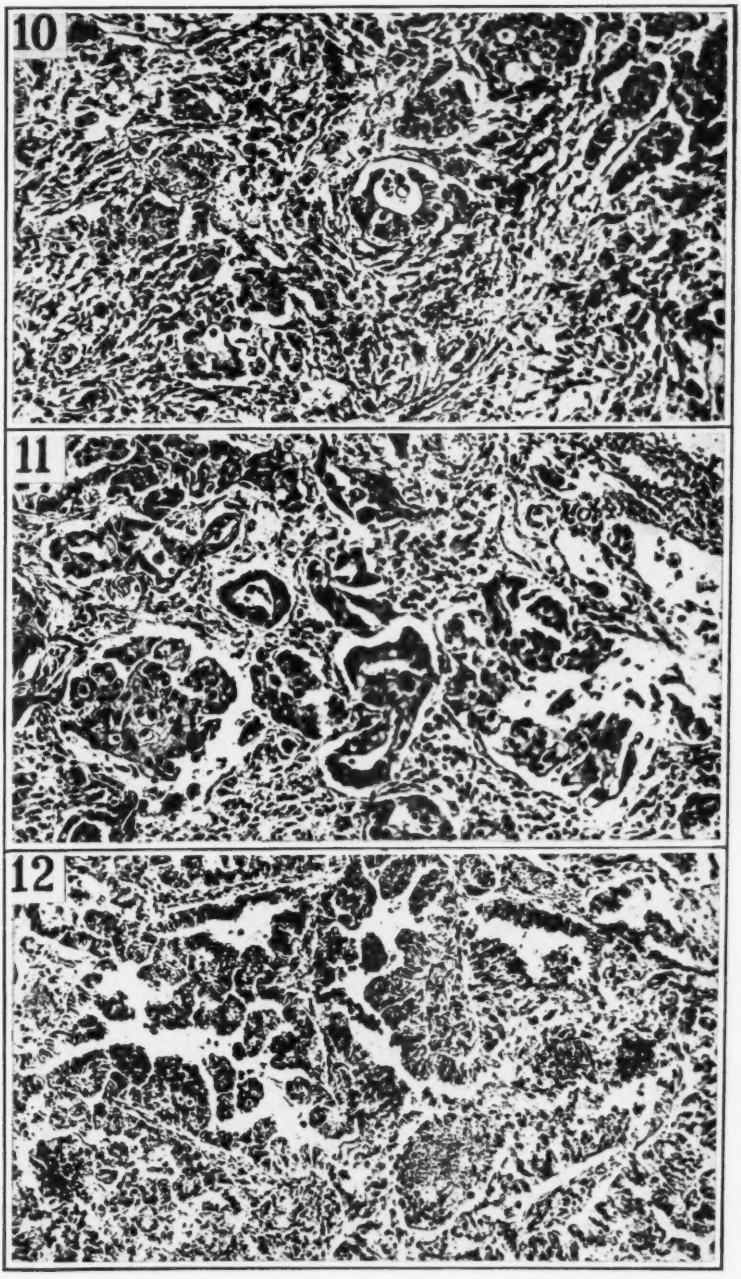


D'Aunoy, Pearson and Halpert Carcinoma of Lung

PLATE 98

FIGS. 10 and 11. Columnar cell carcinoma. The columnar or cuboidal cells, in acinar or tubular arrangement, simulate in a haphazard way normal epithelial structures of the air passages (C 12 and C 2).

FIG. 12. Columnar cell carcinoma. Tall columnar cells are mounted on connective tissue stalks in a papillary arrangement (C 11).



D'Aunoy, Pearson and Halpert Carcinoma of Lung

SCIENTIFIC PROCEEDINGS OF THE THIRTY-NINTH ANNUAL MEETING

OF THE

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

HELD AT RICHMOND, VIRGINIA

APRIL 6TH AND 7TH, 1939

BUSINESS MEETING

OF

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Held in the Library, Medical College of Virginia, Richmond, Virginia

April 6, 1939

VICE-PRESIDENT WELLER PRESIDING

On nomination of the Council the Association voted to instruct the retiring member of the Council, Dr. William Boyd, to cast the ballot for election of the following officers:

President	CARL V. WELLER
Vice-President	STANHOPE BAYNE-JONES
Treasurer	FRANK B. MALLORY
Secretary	HOWARD T. KARSNER
Incoming Member of Council	WILEY D. FORBUS
Assistant Treasurer	FREDERIC PARKER, JR.
Assistant Secretary	FRANCIS BAYLESS

On nomination of the Council the Association voted to elect Dr. Alan R. Moritz to fill the unexpired term of Dr. Earl B. McKinley. This term expires in 1942.

Voted to elect the following new members:

Archie H. Baggenstoss
Orville T. Bailey
Harvey P. Barret
Oscar O. Christianson
Dale Rex Coman
Warren C. Corwin
Oran I. Cutler
Ralph L. Ferguson
Harold Fink
John W. Hall
Francis F. Harrison
George M. Hass
Milton Helpern
Arthur T. Hertig
Russell L. Holman
Cornelius A. Hospers
John S. Howe
George W. Jones

Lester S. King
Kurt E. Landé
Edwin H. Lennette
Victor Levine
Fritz Levy
Averill A. Liebow
Leon S. Lippincott
David G. Mason
William L. McNamara
Arthur A. Nelson
Robert J. Parsons
S. Milton Rabson
Alex B. Ragins
Jacob M. Ravid
Philipp Rezek
Paul S. Rhoads
Walter Schiller
Joseph Schleifstein
Hans Smetana
Edith E. Sproul
Joseph Victor
Emory D. Warner
William B. Wartman
Jarrett E. Williams

Voted to accept with regret the resignations of Drs. M. F. Boyd, A. G. Ellis, F. R. Sabin, E. McD. Stanton, R. P. Strong and W. H. Watters.

Voted to record with deep regret the deaths of Drs. W. H. Chase, W. C. Johnson, H. A. McCordock, E. B. McKinley, and M. J. Sittenfield.

The Secretary announced that the next meeting of the Association will be held at the University of Pittsburgh, Pittsburgh, Pennsylvania, March 21 and 22, 1940.

The Secretary announced that the Symposium for next year will be on the subject of the Pathology of Vitamin Deficiencies and that there will be no formally selected referee.

The Secretary read the following resolution, adopted by the Council:

Since the agreement between the American Chemical Society, the American Medical Association and the American Association of Pathologists and Bacteriologists was consummated in 1924, the policy of the American Association of Pathologists and Bacteriologists has changed in the direction of restricting its activities to the "advancement of the knowledge of disease." For this reason the American Association of Pathologists and Bacteriologists, without prejudice, withdraws from the agreement.

The Secretary announced that in the future contributions to the program, including illustrative material, shall be presented within a limit of 10 minutes, except in the case of invited guests.

Voted to authorize the publication in the Scientific Proceedings

of the Association abstracts of papers listed in the program as "Read by Title."

The Secretary then proposed to the Vice-President that the members and guests of the Association stand for a moment's silent tribute to the memory of Dr. Earl B. McKinley.

The scientific session proceeded as in the following program.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

PATHOGENETIC STUDIES OF TUBERCULOUS LESIONS IN ADULTS. Kornel L. Terplan, Buffalo, N. Y.

Abstract. Among 267 adults between 19 and 80 years of age recent primary tuberculosis was found in 33, two typical tuberculous complexes (Ranke) of different ages (one healed, the other recent) in 22, and a recent caseated complex in the presence of a healed primary focus without corresponding lesions in the lymph nodes was found in 7 cases. In addition a primary focus without gross or microscopic evidence of tuberculosis in the regional lymph nodes was present in 20 cases. Recent focal tuberculous lesions, as incidental findings, mostly in one subapical area, in the presence of a healed stony complex, were found in 17 cases. In none of these were hematogenous lesions observed. In 13 cases with a recent caseated complex as an incidental finding one or more additional caseated foci in subapical fields or in lower lobes were seen which had the same histological structure as the primary focus. There was no evidence of hematogenous seeding in these cases. Chronic pulmonary tuberculosis with intrabronchial spread of the well known picture of the so-called reinfection type was present in 18 cases. In all of these, remnants of an old first infection, namely an ossified or stony complex, were demonstrated. In 8 cases the lymph node groups regional to the progressive postprimary pulmonary lesions showed marked tubercular lesions similar to those seen in lymph nodes adjoining an active Ghon focus of first infection. The anatomical picture in the majority of the cases of this group pointed to exogenous reinfection or superinfection of the lungs. In 2 instances a healed stony complex of primary intestinal tuberculosis was found with an overwhelming recent tuberculous bronchopneumonia with extensive intrabronchial spread. This was obviously an effect of true exogenous reinfection. In 1 case only the anatomical picture suggested a continuous progressive lymphogenic spread in direct connection with a primary focus. In this case the lower tracheobronchial lymph nodes, those in the posterior mediastinum and on each side of the abdominal aorta, including especially the celiac nodes, showed extensive caseation and direct spread into both adrenal glands, causing Addison's disease.

Of the 33 cases with a relatively recent complex, in 15 progressive tuberculosis with hematogenous or intrabronchial spread had developed. In only 3 cases out of 28, progressive hematogenous or intrabronchial tuberculosis was caused by the recent complex of a secondary infection.

Many incidental findings in such cases where death was not caused by tuberculosis are of considerable significance for a better understanding of the pathogenesis of tuberculous lesions. This applies especially to the apical and subapical foci. Our material contains many cases in which these mostly single focal lesions had formed during the active stage of the primary focus. As there was no anatomical evidence of hematogenous metastases to other organs these single foci had developed either following superinfection from without or extension from the primary focus by intrabronchial spread. The

histological structure of these additional foci was identical with that of the primary focus. Cases in which primary tuberculous infection remained restricted to the parenchyma of the lungs without even microscopic evidence of spread to the lymph nodes formed about 10 per cent of our material. The incidence of true exogenous reinfections of the lung with the formation of a typical tuberculous complex of Ranke in the presence of an old, usually completely healed complex was also around 10 per cent of our material thus far examined.

Discussion

(Dr. Esmond R. Long, Philadelphia, Pa.) I should like to ask if Dr. Terplan has the impression that the later primaries, even though they do involve the lymph nodes, involve them to a considerably less extent than primary tuberculosis of childhood. It is quite obvious that as we are entering a period with less and less primary infection in the earlier years, primary infection is being postponed. We have not yet studied the question enough to know exactly how it compares when first acquired in adult life with the primaries which were formerly so frequent in childhood. Postmortem material is of course selected material, in that, at least, the patient dies. There is a definite clinical impression in some quarters that late primaries behave differently from the early primaries. The early primaries, as a rule, show distinct changes in the lymph nodes. The primary infections we now see in nurses and in medical students seem to lack changes in the lymph nodes which can be seen in the x-ray film. I have no doubt these changes could be seen in the hilum nodes if we could dissect them out. It would seem as though the primaries of later life are different from those of childhood.

(Dr. Béla Halpert, New Orleans, La.) What criteria were used in differentiating morphologically between the early and the more recent lesions — those which are fairly close to one another?

(Dr. William H. Feldman, Rochester, Minn.) I should like to ask if the definite tuberculous character of the primary lesions was established by guinea pig inoculation.

(Dr. Terplan, closing.) In reply to Dr. Long's question, I should like to say this: Kuess, and later Blumenberg, are of the opinion that the tuberculous changes in lymph nodes regional to a focus of late primary infection are much less marked than those seen in primary complexes in children. The material we have examined so far during the past 7 years does not support that view. As a matter of fact, the changes in the lymph nodes in many cases of decidedly late primary infection in individuals from 20 to 40 years of age, and even in one senile patient over 80, which were found incidentally at postmortem, were just as marked as those in children who did not die of tuberculosis. I believe that age alone is not the guiding principle in determining the extent and degree of lesions of the lymph nodes regional to the primary tuberculous focus.

In reply to Dr. Halpert, the criteria we apply for differentiating between older and more recent foci are of course only relative. A focus completely encapsulated in the calcified or partially ossified state is much older than a caseated lesion. On the other hand, we could not find any principal difference in the structure of foci of first infection and focal lesions of so-called reinfection. We were not able to support the claim of Puhl and Aschoff that single foci of reinfection always show a structure different from that of pri-

mary foci. We have shown, especially in complexes of different ages, that the focus of the second infection has exactly the same histological structure as that of the first, and that single, focal subapical lesions can look exactly like a typical primary focus with a narrow bony ring, exhibiting in sections stained for elastic fibers a caseated pneumonic structure. We are well aware that not all calcified structures, such as can be found in children, and more so in adults, are of tuberculous nature. We call a calcified focus tuberculous only if we can detect the original caseated pneumonic alveolar pattern.

In reply to Dr. Feldman, we examined many old tuberculous foci for acid-fast bacilli. We have not used calcified or ossified foci for animal inoculation. The more recent literature on this subject reveals the fact that in well controlled experiments of this kind no evidence of living tubercle bacilli in such obsolete foci was obtained. I cite only the experimental work of Schrader. All his injections gave negative results. Not even in primary foci in the chalky state did we succeed in demonstrating tubercle bacilli. We examined a number of these cases as we were interested to determine whether or not true reinfection can occur in the presence of a chalky focus. If this should be a possibility, morphologists would be inclined to speak of superinfection rather than of true reinfection. We have examined chalky foci found in children without demonstrating tubercle bacilli. In some of these cases, however, in addition a recent caseated complex of a second exogenous infection of the same anatomical type as that seen in the primary complex was found in the other lung.

THE INCIDENCE AND SIGNIFICANCE OF HEALED MILIARY TUBERCLES IN THE PARENCHYMATOUS ORGANS. Herbert S. Reichle and (by invitation) John L. Work, Cleveland, Ohio.

Abstract. Small spherical bodies, usually called phleboliths, were found in the liver, spleen and kidneys in 20 per cent of 500 consecutive autopsies at the Cleveland City Hospital. No condition other than parenchymatous degeneration, hyperemia and edema, primary tuberculosis and arteriosclerosis occurred in more than 20 per cent of the autopsies. In 94.5 per cent of the cases with nodules, primary or reinfection tuberculosis, healed or active, was found; in only 5 cases was evidence of a tuberculous infection lacking, and in only 1 of these had the lungs been examined by x-ray. The nodules were histologically indistinguishable from small primary tubercles or healed primary satellite tubercles and tubercle bacilli were demonstrated by animal inoculation in 3 out of 14 cases. They were found in the parenchyma of the spleen, under the capsule of the liver and in the cortex of the kidneys, in locations where large veins are not found. True phleboliths, on the other hand, were demonstrated in the hilum of the spleen, also in the periprostatic veins, and their structure and relation to surrounding tissues were entirely different. These lesions are therefore to be regarded as miliary tubercles, the result of a hematogenous dissemination during the primary phase of infection. This statement is further strengthened by the fact that the incidence of these tubercles is the same throughout all age groups of our population.

In order to study a possible relation of this hematogenous dissemination to the resistance of the individual, the cases were further divided into three groups. Group I consists of cases with obsolete tuberculous lesions showing no activity microscopically; the bulk of these cases showed only one or more

components of a primary complex and this group is designated as "resistant." Group II includes the cases in which there was no clinically significant disease but in which microscopic study showed some activity; this group is designated as "moderately resistant." Group III comprises cases presenting clinically important active disease and the majority of these patients died from tuberculosis; this group is designated as "susceptible." The incidence of healed miliary tubercles in these groups was 39.2, 23.5 and 17.2, respectively, and this relationship obtains for all significant statistics in subgroups arranged according to age and color.

It is therefore concluded that the lesions described represent the result of an early hematogenous spread with limited seeding of tubercles. This acts as an autovaccination, conferring upon such individuals a resistance against reinfection which others, not showing the results of such a spread, do not have.

Discussion

(Dr. Alfred Plaut, New York City.) I should like to know how often in this autopsy material the parasitic nodules in the liver which we are accustomed to call *Pentastoma denticulatum*, or *Linguatula rhinaria* were found. In New York I find these small nodules rather frequently, and they bear a striking resemblance to some of the pictures shown to us here. This especially refers to the liver. I have had no experience with similar nodules in the kidney, and I have seldom seen them in the spleen.

(Dr. Kornel L. Terplan, Buffalo, N. Y.) The lesions in the spleen and liver which Dr. Reichle has shown have been found quite frequently in our postmortem material. It is my impression that they are seen more frequently here than we had seen them abroad at the Institute of Dr. Ghon. We feel as Dr. Reichle does that the majority of such calcified nodules in the spleen are tubercles and not phleboliths. We have seen these tubercles, especially in cases with hematogenous tuberculosis, in other organs. I would prefer to speak of them as hematogenous tubercles rather than as miliary tubercles. With regard to the significance of these tubercles in relation to an increase in the resistance against reinfection, we had some experience different from that of Dr. Reichle. We have seen healed calcified tubercles of the same structure as those demonstrated by Dr. Reichle in the spleen, together with remnants of an old primary complex. In some of these cases, however, there was evidence of a true exogenous reinfection of more recent nature. We find it difficult to interpret our morphological lesions in terms of different degrees or changes of "resistance." This applies especially to children. They might acquire a tuberculous lesion, a single focus, restricted completely to the parenchyma of the lung, with no spread to the regional lymph nodes. This lesion heals completely. It might remain the only tuberculous lesion throughout life. In other cases, however, in children with a similar single healing or healed focus, recent caseated lobular pneumonic foci were found in addition with masses of tubercle bacilli, spreading by the intrabronchial route.

(Dr. Morton McCutcheon, Philadelphia, Pa.) Dr. Reichle's first slide showed a good many of these little lesions in the tissue. I wonder whether he would think that solitary lesions, 2 to 3 mm. in size, in the spleen or in the liver also belong in this classification of obsolete tubercles.

(Dr. Benjamin J. Clawson, Minneapolis, Minn.) I should like to ask whether a tuberculin test was done on these patients, especially those in Group I, and if so whether it was positive or negative.

(Dr. Otto Saphir, Chicago, Ill.) Has Dr. Reichle seen a case which showed both lesions — calcified lesions in the liver and spleen, and recent miliary tubercles?

(Dr. Paul R. Cannon, Chicago, Ill.) I should like to ask Dr. Reichle if he has seen or heard of not only calcified but ossified lesions in the spleen. Huggins has been unable to produce experimental ossification in the spleen. I wonder if you know of any instance of ossification of such nodules in the spleen.

(Dr. Virgil H. Moon, Philadelphia, Pa.) We are all familiar with the occasional occurrence of a progressive destructive organ tuberculosis, a sequel of the primary complex, which causes destruction of the adrenals, kidneys, and sometimes the liver, spleen and brain. I wonder whether Dr. Reichle would interpret the lesions he has described, and which we have all seen, as possibly healed extrapulmonary lesions of the primary tuberculous complex which, had they not healed, would have progressed to destructive caseous lesions in one organ or another.

(Dr. Reichle, closing.) Any attempt to go into all these questions in detail would be impractical. Let me assure you that I realize the difficulties, whenever a discussion concerning resistance in tuberculosis is opened. The paper I presented is more or less of a thought and not a conclusion. This problem has to be attacked from so many different angles that any attempt to solve it from one angle is quite hopeless and leads to a false perspective.

The next point I should like to call attention to is that when I talk about resistance I am obviously talking about it as a statistical problem, and on this basis there will be individual cases which always fall out of the scheme. There is no doubt about that. Whenever we talk about resistance in tuberculosis we talk about a very limited and very variable factor, and we shall find that there is evidence coming from all sides pointing to the fact that resistance to tuberculosis depends on this paradox: the spread of elements of the primary infection more or less throughout the body, without the development of tuberculous septicemia, *i.e.* miliary tuberculosis. It is my personal opinion that a primary process remaining localized will never give the resistance that a spreading one will. That was shown in Calmette's work and by a number of others. Furthermore, experimental work has shown that spread occurs very early. This work I believe was done in Saranac, and showed that if tubercle bacilli are injected into the pad of a guinea pig's foot, and amputation of the extremity is performed within a few hours, the organisms would nevertheless spread to the rest of the body even though no primary focus had yet been established. Therefore, although I agree that there are cases which will not possibly fit into this scheme, I do think that in general the spread is part of the defensive mechanism. I call your attention to the old rule of pathologists that we do not find many cases of miliary tuberculosis in individuals with extensive organ disease. Individuals with chronic pulmonary tuberculosis coming from sanatoriums do not usually show miliary tuberculosis, and on the other hand, patients with miliary tuberculosis usually have a rather small focus of disease somewhere in the body.

Concerning Dr. Plaut's question, we have seen parasites in the liver but do not believe these lesions are the result of a parasitic involvement. Possibly a parasitic infestation might heal in such a fashion.

We have seen cases where there were only one or two, or perhaps a single tubercle, in the spleen. Let me call your attention to the fact that all calcified

lesions are not tubercles. In every single case some of the lesions were examined microscopically, and if we found the architecture described, then we included the case in our group.

To. Dr. Clawson I regret to say that we have no knowledge concerning the tuberculin test. These were routine autopsies.

Dr. Saphir asked about the combination of miliary tuberculosis and these lesions. We have 2 cases in children with progressive primary tuberculosis who showed fresh tubercles and these older miliary lesions. Dr. Terplan is justified in objecting to the term miliary. Hematogenous would be better.

To the best of my knowledge we did not see ossified tubercles in the spleen; they were, however, found in the liver.

In reply to Dr. Moon, permit me to emphasize that we regard these lesions as instances of hematogenous tuberculosis.

THE OCCURRENCE OF VIRULENT TUBERCLE BACILLI IN PRESUMABLY NON-TUBERCULOUS TISSUES OF THE LUNG. William H. Feldman and (by invitation) A. H. Baggenstoss, Rochester, Minn.

Abstract. Opie and Aronson having reported the demonstration of virulent tubercle bacilli in the presumably non-tuberculous lung tissue in 15 of 33 bodies examined in Philadelphia in 1927, a comparable study was made of material secured at Rochester, Minnesota. Tissues from 51 unembalmed bodies were utilized for the inoculation of guinea pigs. The age distribution was from 2½ to 93 years, with the largest number of cases in the fifth and sixth decades. All were white. Thirty-four were males and 17 females. The bodies selected for the study represented individuals who with one exception had died of causes other than tuberculosis. In 12 of the bodies no gross or microscopic evidence of tuberculosis was found, while in 38 there were lesions of latent or healed tuberculosis. In the majority of instances the signs of primary tuberculosis present were those of the primary complex of the lungs.

Material for the inoculation of guinea pigs consisted of what appeared to be non-tuberculous portions of the upper and of the lower lobes of each lung and the apparently non-tuberculous hilar lymph nodes. In all but 3 cases three emulsions of tissue were prepared from each body and used to inject 6 guinea pigs. A total of 150 emulsions was utilized to inject a total of 300 animals.

Positive results were obtained from only 3 cases and since in 1 of these the cause of death was tuberculous enteritis and peritonitis, only 2 positive cases need be considered. In 1 case tubercle bacilli definitely identified as bovine in type were obtained from the spleen of 1 of 2 guinea pigs previously inoculated with a composite emulsion prepared from presumably normal tissues from the parenchyma of the upper and lower lobes of the left lung. In another case tubercle bacilli were demonstrated from the hilar lymph nodes.

The results of this study made of material from an area where the tuberculosis morbidity is not high indicate that virulent tubercle bacilli are infrequently present in the presumably non-tuberculous tissue of the lungs of individuals dying of causes other than tuberculosis.

Discussion

(Dr. Hans P. Popper, Chicago, Ill.) I should like to ask whether in these cases the blood and other organs were examined for tubercle bacilli. While

in Vienna we examined the blood, spleen, liver and kidneys in cases of fatal and non-fatal tuberculosis, and were able to prove fairly often the presence of tubercle bacilli by guinea pig inoculation and culture.

(Dr. Kornel L. Terplan, Buffalo, N. Y.) I should like to add that the positive result in 1 case of Dr. Feldman's, in which tuberculous enteritis was present, is in line with a number of experiments we carried out after injecting material from lymph nodes from cases of Hodgkin's disease. Although such nodes had not exhibited tuberculous lesions, we produced tuberculosis in guinea pigs whenever they were from cases in which marked active tuberculosis was combined with Hodgkin's disease, especially in the fulminating miliary type seen in adults. With regard to the other positive result in Dr. Feldman's material, I am inclined to believe that its source might have been a small active tuberculous lesion in a bronchomediastinal lymph node. These tuberculous lesions in bronchomediastinal lymph nodes are quite frequently found in older individuals. It was thought that they developed and spread following lymphoglandular exacerbation. Sometimes they are small enough to be overlooked with the unaided eye.

(Dr. Herbert S. Reichle, Cleveland, Ohio.) I would like to emphasize what Dr. Terplan says. I think possibly the difference between the results obtained by Feldman and by Opie are due to the fact that there may have been quite a number of patients with endogenous reactivation in Opie's group. I agree with Dr. Terplan. In the City Hospital we see an unusual number of cases of tuberculosis in individuals who come from the Psychopathic Division or the Tumor Division. I think that the endogenous source of tubercle bacilli cannot be excluded.

(Dr. Feldman, closing.) In reply to Dr. Popper, we did not examine the blood or other organs. We limited the investigation entirely to the lungs and the contiguous lymph nodes.

STUDIES OF THE CHEMOTACTIC PROPERTIES OF TUBERCULOPHOSPHATIDE AND TUBERCULOPOLYSACCHARIDE. William B. Wartman and (by invitation) E. S. Ingraham, Jr., Cleveland, Ohio.

Abstract. In experiments reported before this society 2 years ago it was shown that *in vitro* tuberculoprotein strongly attracted human polymorphonuclear leukocytes. In the present experiments the chemotactic properties of the phosphatide and polysaccharide fractions of the tubercle bacillus were studied. In concentrated form the tuberculophosphatide was found to be toxic for both human and rabbit neutrophils, but in suitable dilution it caused weak attraction of these cells. The tuberculopolysaccharide caused weak negative chemotropism of the leukocytes.

Discussion

(Dr. Morton McCutcheon, Philadelphia, Pa.) I wonder whether Dr. Ingraham has any information about the adsorption of these carbohydrates on kaolin. That is of quite considerable interest to us.

(Dr. Esmond R. Long, Philadelphia, Pa.) Did Dr. Wartman and Dr. Ingraham carry out experiments with leukocytes in both normal and tuberculous animals, and if so, I would be interested to hear if there was a difference in the results.

(Dr. Ingraham, closing.) We did not mean to claim the polysaccharide was

adsorbed. We only used the inert substance as opaque material, and the particles were not washed. They were merely saturated with dilute solution.

In reply to Dr. Long, we did not use tuberculous animals.

A Study of Purified Antigens in Relation to Virulence of Rough and Smooth Strains of Salmonella Aertrycke. G. M. Mackenzie and (by invitation) R. M. Pike and R. E. Swinney, Cooperstown, N. Y.

Abstract. From 2 rough and 2 smooth strains of *Salmonella aertrycke* polysaccharides were prepared by the method of Raistrick and Topley. One of the smooth strains is highly virulent, the other is of very low virulence. The rough strains are avirulent. The polysaccharides from the 2 smooth strains have the same toxicity and immunizing power for mice; analysis of their antigenicity shows them to be complete antigens and serologically indistinguishable. The polysaccharides from the rough strains are much less toxic and possess little or no immunizing power; they too are complete antigens. The polysaccharides from the smooth strains and those from the rough strains show complete antigenic disparity. The results indicate: (1) the smooth somatic polysaccharide is not the major determinant of virulence in *Salmonella aertrycke* infection in mice; (2) in confirmation of the work of Raistrick and Topley, and that of Boivin and collaborators, however, the smooth somatic polysaccharide has been found to be chiefly responsible for immunizing power; (3) the virulence of this species of microorganism is probably determined by serologically inactive components of the bacterial cell; and (4) a polysaccharide, which is also a complete antigen, is present in rough avirulent strains of *Salmonella aertrycke.*

Discussion

(Dr. A. B. Wadsworth, Albany, N. Y.) Was there much difference in the nitrogen content — total and amino — in the rough and smooth preparations?

(Dr. Mackenzie, closing.) Quantitative analyses have been made of these preparations; the nitrogen content is 4.5 to 5 per cent. On hydrolysis the yield of glucose is about 40 per cent from the smooth strains, and 20 per cent from the rough strains. There are some differences in the quantitative elementary analysis, but the chief difference between the smooth and the rough strains is in the yield of glucose on hydrolysis.

A Study of Two Strains of B. Violaceus Isolated from Human Beings. Malcolm H. Soule, Ann Arbor, Mich.

Abstract. A strain of *B. violaceus* was isolated in pure culture at autopsy from the blood, spleen and liver of a girl 15 years of age. Nine months before death a large fluctuating mass in the right side of the neck had been treated surgically and *B. violaceus* was the only viable organism in the aspirated pus. The incision did not heal and roentgen therapy over a period of months merely held the lesion in check; it continued to drain although the patient was otherwise in excellent physical condition. An exploratory biopsy was diagnosed as mixed tuberculous and pyogenic infection. Clinically the case was one of tuberculous adenitis. Cultures and guinea pig inoculations were negative for the tubercle bacillus. A series of tuberculin tests was nega-

tive. Fluoroscopic examinations and x-rays of the chest were negative. Repeated blood cultures were negative; the blood serology was negative for syphilis, *B. abortus*, and *M. melitensis*, *B. tularensis*, *B. typhosus*, and *B. paratyphosus A* and *B.* The report on the gross pathology showed active chronic tuberculosis of the base of the left lung, tuberculous abscesses of the left leaf of the diaphragm; a large chronic tuberculous abscess of the left hypochondrium; an extensive hematogenous chronic tuberculous abscess of the liver; and necrotizing miliary tuberculosis of the spleen, liver and lungs. The histological studies revealed no tubercles, only a septicopyemia.

A comparative study of the morphology, cultural characteristics, biochemical reactions, serology and pathogenicity of this human strain, labelled Human Strain 1, with two old laboratory strains of *B. violaceus* was completed when Black and Shahan reported the isolation in pure culture of a similar organism from purple pustular areas surrounding anthrax-like lesions on the arms and chest of a boy of 6 years. After 2 months these lesions apparently healed and the patient was lost sight of until 13 months later when he appeared with a severe cervical adenitis, high fever and marked prostration. The adenitis subsided and the body became covered with lesions ranging from minute vesicles to large gangrenous areas. Death occurred 15 months after the original observation. Unfortunately no autopsy was granted. The only organism ever found was *B. violaceus*. The same serological and cultural studies mentioned in connection with the young girl were carried out by Black and Shahan with identical results. In addition, they found the blood serum of the body agglutinated the homologous strain of *B. violaceus* in a dilution of 1:1280.

The Shahan strain was added to the comparative studies under the designation Human Strain 2. Morphologically the four strains were identical. There were cultural differences particularly as regards the resistance to aniline compounds. H 1 was sensitive to the concentrations of dyes ordinarily employed in mediums for the isolation of acid-fast organisms; the others were not. A deep violet color was associated with the growth of each strain. All bacilli in a given culture did not produce the same amount of pigment. Strain-specific antibodies were elicited by the injection of rabbits with suspensions of the dead cells. H 2 induced a progressive infection on intraperitoneal injection in rabbits and guinea pigs. Broth suspensions of the four strains were toxic for animals in quantities of 0.5 cc.

In consideration of the aforementioned observations, the wide distribution of *B. violaceus* in air, soil and water should be envisioned as potentially dangerous. If found in diseased processes, this organism should be carefully investigated rather than treated as a contamination.

Discussion

(Dr. Carl V. Weller, Ann Arbor, Mich.) I was responsible for the incorrect diagnosis of mixed tuberculous and pyogenic infection which was made on the biopsied lymph node of this patient, and being fully aware of the subsequent bacteriological investigation, I have reviewed these slides from the standpoint of pathology and cannot see how I could have made any other diagnosis. We have, however, stained not only the same blocks of tissue, but many others from the autopsy in this case, and we have failed to demonstrate acid-fast bacilli by staining tissue sections. As the matter now stands, it is a new entity to me in my experience as a pathologist.

(Dr. Clayton E. Royce, Jacksonville, Fla.) The *B. violaceus* in the 1st case described was found in my laboratory at St. Vincent's Hospital in Jacksonville, and as the author said, we did not feel it would be justifiable to connect it with the disease present in the young woman, although it was found several different times in culture, and no other organisms were demonstrated. I am very glad to be here and to hear the careful analysis which subsequently was made of the organisms recovered from the patient.

(Dr. Howard T. Karsner, Cleveland, Ohio.) I noticed in the autopsy report in the 1st case, that of the young girl, that there was a gross anatomical diagnosis of tuberculosis, and that it was not confirmed by microscopic examination. I think it would be of interest if we knew something about the microscopic appearance of the nodules which in gross appeared to be tuberculous.

(Dr. Paul Klemperer, New York City.) Was the organism found in the tissues? The question which Dr. Karsner asked would be interesting to hear about.

(Dr. Soule.) I can comment on Dr. Royce's remarks, namely that the bacteriological work done in his laboratory was most thorough and of the highest quality. In our own studies *B. violaceus* was never found until autopsy because the pus and biopsy specimens were treated with 4 per cent potassium hydroxide previous to the inoculation of laboratory mediums. This is the routine technique in the isolation of the tubercle bacillus. We also found this strain sensitive to the dyes employed in acid-fast mediums. This was not true of the other strains investigated. The other questions I cannot answer.

(Dr. Weller.) I am sorry I have not refreshed my mind recently enough to give the detailed answer to these questions that they deserve. In the first place I think the prosector at the time of the autopsy was somewhat influenced by the biopsy diagnosis, so that he was quite satisfied with the diagnosis of generalized tuberculosis.

(Dr. Frank B. Lynch, Philadelphia, Pa.) Was there any study of this tissue made from the standpoint of *Coccidioides immitis*? Dr. Soule answered the question that it was not cultured in his laboratory. I wonder if that has been thought of in a study of the tissues.

(Dr. Weller.) With the very complete study we made of this case, if there had been *Coccidioides immitis* we would have been aware of that fact. There was no indication whatever of *Coccidioides immitis* infection. We are quite aware of the necessity of looking into that group in all the tuberculous-like material that comes to our department.

(Dr. Karsner.) Mr. President, could your remarks in regard to the microscopic appearance be expanded a little when the material is ready for publication in the Proceedings?

(Dr. Weller.) I will be glad to cooperate with Dr. Soule in that respect.

(*Prepared Statement by Dr. Weller*)

"The first biopsy specimen showed an area of combined suppuration and caseous necrosis. There was no tubercle formation but the border of the necrotic area was composed in part of epithelioid cells with many large mononuclear phagocytes. This zone was relatively avascular. It was thought that even in the absence of definite tubercles, a diagnosis of mixed tuberculous and pyogenic infection was justified. A second biopsy, taken about 1 month later, showed substantially the same changes, but with even more caseous

necrosis. At the autopsy, focal necrotizing lesions were found in the lungs, spleen, liver and pelvic peritoneum. The prosector made a diagnosis from the gross appearances only of active chronic tuberculosis of the base of the left lung, chronic tuberculous abscess of the left hypochondrium and generalized miliary tuberculosis. Microscopic examination showed these lesions to be essentially pyemic abscesses, but even microscopically the purulent exudate and tissue débris in the interior of each of them showed changes closely approaching caseous necrosis. The borders of the abscesses were made up of granulation tissue which was but slightly vascular and rich in large mononuclear phagocytes. No tubercles were found and no multinucleate giant cells. The various higher fungi were searched for and none was found. Staining for tubercle bacilli gave negative results on the biopsy material and also on selected blocks from the material obtained at autopsy. Likewise cultures for tubercle bacilli were negative, as was also animal inoculation. The recovery of *B. violaceus* from blood from the heart, from exudate from the lesions in the neck, and from pyemic abscesses in the liver has been described by Dr. Soule."

METHODS WHICH MAY AID THROUGH CORRELATION IN EXPLAINING PHYSIOLOGICAL AND PATHOLOGICAL SEQUENCES OF EVENTS IN THE SPREAD OF INFLAMMATION. John W. Williams, Cambridge, Mass.

Abstract. New methods (*Am. J. M. Technol.*, May, 1939, **5**, 68–71) show a decrease in Eh for 45–90 mm. in depth in gel mediums autoclaved and solidified in 16 by 200 mm. test tubes and set aside for periods up to 2 weeks (0.08 to −0.15v. from top to 45 mm. 1 minute after insertion of electrodes, using a Beckman potentiometer with a calomel half cell reference electrode and a medium containing 2 gm. nutrient broth Difco, 7.5 gm. agar, 1 gm. dextrose, 0.48 gm. sodium hydroxide, 1000 cc. water). Controls of agar and sodium hydroxide show these can account for the greater part of the Eh change. More acid mediums shift the curve generally into a more positive Eh range. The pH does not show significant variation with depth (8.3–8.4) in above. At depths greater than 45 mm. the curve shows some fluctuation, but not a decrease trend. Mediums placed under 60 pounds of nitrogen 4 days show a slightly greater Eh (0.11 to −0.13v. from top to 45 mm.) and slightly greater decrease in pH (7.8–8.36). Mediums placed under 60 pounds of oxygen 4 days show a greater shift of the curve to the right or a more positive Eh (0.226 to −0.142 from top to 75 mm.) and a decrease in pH (7.2–8.74). Mediums placed under 60 pounds of carbon dioxide show shift to the right but lower Eh than oxygen (0.15 to −0.128v. from top to 90 mm.) with greater decrease in pH (6.0–8.34): there seems a more gradual decrease in Eh with possibly greater penetration of carbon dioxide possibly related to decrease in acidity.

Change with nitrogen and carbon dioxide in large part may be dependent on the increase in acidity. Greater increase in Eh under oxygen indicates an effect of oxygen independent of pH. The importance of penetration of gas and resistance to this is indicated by the fact that the low Eh noted in mediums in the atmosphere is eventually reached in tubes placed under carbon dioxide and oxygen. In previous papers (*Am. J. M. Technol.*, 1938, **4**, 58–61; *Am. J. Path.*, 1938, **14**, 642–645; and *Growth*, 1939, **3**, 21–33) variations in the depth of growths of microorganisms in shake cultures corre-

sponding to Eh and oxygen tension variations described here were reported. Gelatin and horse blood clot also show some variation of Eh; consistent and experimentally sufficiently marked effects have not yet been demonstrated for an inorganic gel such as silica gel.

Since our bodies are fundamentally organic gel in consistence this work suggests the gel may function as a tramway for a gradient of Eh and possibly pH. It may serve as a structure on which variations in physiology and pathology occur. While the gas tensions used are large, smaller variations between the blood stream and metabolizing tissues, and between normal and pathological tissues may be assumed to act likewise. Just as bacteria (anaerobes and aerobes) when planted in shake cultures grow in positions where physicochemical conditions for growth are optimum, so the cells of our body may select optimum and appropriate sites for growth. Various diseases modify conditions which the body attempts to remedy. Since practically all appearances produced by the colloidal chemist by inorganic precipitates in gels have been produced by growth of microorganisms, a suitable site dependent on physicochemical conditions and essential nutrient for proliferation and existence of our cells can be logically postulated.

A Study of Experimental Nephritis in the Horse. Joseph Schleifstein (by invitation), Albany, N. Y.

Abstract. A study has been made of the renal lesions of 168 horses under experimental conditions of immunization with toxic injections of bacterial cultures and toxins in the production of therapeutic serums.

The changes in the kidney under certain conditions correspond to the following lesions observed in man: (1) acute glomerular congestion, (2) acute diffuse glomerulonephritis, and (3) focal or embolic glomerulonephritis.

(1) Acute glomerular congestion was observed in horses that died from acute shock a few hours after an injection of bacterial culture or toxin. The glomerular tufts were so distended that they completely filled the capsule and the capillaries were packed with red cells.

(2) The changes in the kidney were those of the acute diffuse glomerulonephritis that occurs in man when the horses lived for from 3 days to 5 weeks after the last injection of bacterial culture or toxin. These acute changes did not progress to chronicity. The horses were relatively highly immunized.

(3) The focal or embolic glomerulonephritic lesions of man as described by Löhlein and Baehr occurred in 16 of 20 animals with endocarditis and bacteremia. All stages of the lesion, from early glomerular necrosis to healing and fibrosis, were observed.

Discussion

(Dr. William H. Feldman, Rochester, Minn.) I am curious to know if the material was examined for amyloid.

(Dr. Irving Graef, New York City.) I should like to ask if Dr. Schleifstein has encountered any of the so-called embolic lesions in animals that did not have endocarditis.

(Dr. Herbert Fox, Philadelphia, Pa.) This paper seems to have two very distinct significances. The fact that glomerular lesions are found under these circumstances is rather unusual for spontaneous nephritis in the group of

animals to which the horse belongs. In the spontaneous nephritis of that group more often it is the parenchyma which suffers and the glomeruli do not, and that seems to strengthen the fact that there is in this experimental procedure some value in the production of nephritis. The secondary stages are much more interstitial, surrounding the tubules rather than around the glomeruli.

(Dr. Paul Klemperer, New York City.) I wonder what was the distribution of the lesions in respect to the organisms used for immunization.

(Dr. Schleifstein, closing.) In regard to Dr. Feldman's question, these kidneys were all carefully stained for amyloid and amyloid was not present.

In reply to Dr. Graef, there was one instance where an embolic lesion was present where there was no endocarditis, but in this there was a thrombosis of the iliac arteries.

I appreciate very much the comment of Dr. Fox on spontaneous lesions.

In reply to Dr. Klemperer, I may state that generally speaking the streptococcus showed the most severe glomerular lesions, then the meningococcus and the pneumococcus. In the toxins the lesions were not so severe as with the streptococcus.

EXPERIMENTAL PERITONITIS: ALTERATION OF THE LEUKOCYTIC RESPONSE AFTER REPEATED INJECTIONS OF PHYSIOLOGIC SALINE. Dale Rex Coman (by invitation), Philadelphia, Pa. (Presented by Dr. Morton McCutcheon.)

Abstract. Experimental peritonitis was produced by the method of de Haan, which consists in injecting physiologic saline into the peritoneal cavity of rabbits; after several hours the fluid is withdrawn and it then contains large numbers of leukocytes, nearly all of them polymorphonuclears. It is not understood why saline solution causes exudation of leukocytes, since sodium chloride is usually not chemotactic for these cells. Experiments were made to find whether exudation is due to impurities in the saline solution, and, more generally, whether emigration of leukocytes is always the result of chemotaxis or, on the contrary, whether it may occur in the absence of chemotactic substances.

One series of rabbits was injected with saline made with distilled water prepared in the ordinary way, in a copper still, and stored in a large tank. Another series was injected with saline made with doubly distilled water, freshly prepared, with every precaution to ensure sterility and purity. None of these rabbits had been injected previously. Cell counts showed small to moderate numbers of leukocytes in the peritoneal fluid of both series of rabbits, and there was no significant difference between counts in the two series.

Several days later the rabbits that had been injected with ordinary saline solution received a second injection, half of them again with ordinary saline and half of them with saline prepared with redistilled water. There was now a striking difference between the two series: rabbits injected with ordinary saline showed a four-fold increase in leukocytes, while those injected with specially pure saline had about the same number of cells as on the first injection. The reactivity of the peritoneal vessels had apparently become increased, so that differences in the effects of the two solutions, masked on the first injection, became obvious on the second. This difference in the effects of the two saline solutions appeared to be due to the presence of chemotactic

substances in the ordinary saline, and absence of such substances in specially purified saline, as demonstrated by experiments *in vitro*. It is concluded that exudation of polymorphonuclear leukocytes may take place in the absence of substances having demonstrable chemotactic effect, but when chemotactic substances are present, emigration is greatly increased.

Discussion

(Dr. A. B. Wadsworth, Albany, N. Y.) This field of study is extremely interesting. Maltaner (*J. Hyg.*, 1921, 19, 309) repeated the experiments of Buchner on positive and negative chemotaxis. He found that irrespective of the agents that were used in the early experiments of Buchner the positive or negative so-called chemotactic reactions took place, simply according to the concentration of the salt solution. I think the experiments were done in capillary tubes under the skin and in the peritoneum, and the leukocytes penetrated the capillary tubes that contained the experimental fluids, or did not penetrate, according to the concentration of salt solution, — whether the density of the solution was greater or less than that of the body fluids.

(Dr. Sheldon A. Jacobson, Brooklyn, N. Y.) I should like to ask what the interval of time was between the two injections. If it was not excessively great, I wonder whether the author would not consider, in addition to his own interpretation, the following incidental factor in the obtaining of these results. Whatever this chemotactic principle was, whether it was an additional substance or saline, it evidently acted as an irritant. The method of fixation by irritation has been very well demonstrated in a series of papers by Menkin, and I wonder whether it is not possible that the first injection fixed the irritation in the lymphatics and local tissues around the peritoneum, so that when the second injection occurred, the irritant was unable to escape and was confined *in situ*, and therefore had a more pronounced effect.

(Dr. McCutcheon, closing.) I thank Dr. Wadsworth for his comments.

In reply to Dr. Jacobson, the time in these experiments between injections varied from 2 days to about a week. However, in Hamberger's earlier experiments he usually allowed 2 weeks to elapse between injections. I think Dr. Jacobson's suggestion is very interesting.

EXPERIMENTAL ARTHRITIS IN MICE PRODUCED BY FILTRABLE PLEUROPNEUMONIA-LIKE MIRCOORGANISMS. Albert B. Sabin (by invitation), New York City.

Abstract. Two strains of filtrable, pleuropneumonia-like microorganisms, recently isolated from mice, were found to possess specific tissue affinities of such a nature that they can give rise to two experimental diseases in mice, which in some respects resemble rheumatic fever and rheumatoid arthritis in man. Strain A can multiply in the brain and in association with the cells of the parietal and visceral peritoneum, pleura and pericardium, with the elaboration of a specific exotoxin, having a special affinity for the cerebellum, which either kills the mice within a few hours or leaves them suffering from a choreiform syndrome. This toxin is also produced *in vitro* in cultures, is heat-labile, and can be neutralized by a specific antitoxin. When the rapid toxic death which usually follows the intravenous injection of the culture is prevented by the use of older mice or by inoculation of centrifuged microorganisms, about 30 to 40 per cent develop a migratory polyarthritis, during

the course of which some of the mice exhibit choreiform signs. Most of the mice tend to recover from the arthritis produced by Strain A.

Strain B, on the other hand, has a specific affinity for the joints (it does not multiply in the skin or subcutaneous tissue, brain, viscera, or their linings), in which it gives rise to a chronic progressive proliferative arthritis which clinically and pathologically bears a marked resemblance to rheumatoid arthritis in man. Arthritis was produced in practically 100 per cent of mice when 0.5 cc. of a 24 hour culture was injected intravenously, or 1 cc. intraperitoneally. It has also been possible to obtain arthritis in a small number of mice which developed a focal infection after inoculation into the vitreous of the eye. After a preliminary phase, during which the arthritis is migratory, the process becomes progressive and chronic in one or more joints, leading to ankylosis (especially in the knees) in about 70 per cent of mice. The affected animals appear otherwise healthy and not one of 150 with joint involvement (B strain) has as yet died of the infection. The microorganism has been cultivated (by the method of "blind passage") from affected joints as late as 70 days after inoculation. Pathological changes are limited to the joints and consist predominantly of proliferation in the synovial membrane, the capsule, and the perichondrium of the articular cartilage, with similar changes in the subchondral epiphyseal marrow. Rabbits and guinea pigs were not susceptible. Filtration through gradocol membranes indicated that the smallest unit of these microorganisms is not much larger than vaccine virus. The two strains possess a common antigen but are immunologically distinct.

Discussion

(Dr. Benjamin J. Clawson, Minneapolis, Minn.) The similarity of the inflammatory reaction shown here is certainly great when compared with that of acute rheumatic fever. I should like to ask Dr. Sabin if he examined the heart valves in these cases.

(Dr. Sheldon A. Jacobson, Brooklyn, N. Y.) Have Dr. Sabin's experiments lasted long enough for him to be able to state whether the disease in these animals shows any tendency to successive periods of remission and exacerbation?

(Dr. S. A. Goldberg, Newark, N. J.) I am very much impressed with the similarity of the lesions shown by Dr. Sabin to those we studied in arthritis in animals in 1917. We found certain strains of *B. coli* and streptococci in the lesions.

(Dr. Carl V. Weller, Ann Arbor, Mich.) Chondroplastic synovial villous processes are seen in human pathology. I remember in the first year or two in which I was in pathology a surgeon brought into the laboratory about a pint of round firm bodies, and with an assumption of learning that was unjustified, I told him they were corpora oryzoidea. When we examined them microscopically, there was not a single rice body; we found only small spherical nodules of cartilage — detached chondroplastic synovial villi. These pictures brought that early experience to my mind.

(Dr. Sabin, closing.) I expected Dr. Clawson's question, and if I may have a minute to show the last two slides which I did not have time for, they might answer his question. I have not yet had time to make a complete study of the manifestations in the hearts of the mice injected with Strain A, but I have found two things which I want to show. Some of the myocardial

vessels exhibit a subendothelial infiltration of this sort (illustrating on screen) with a palisading epithelioid type of cells, which I have not yet observed in the normal, but I am not yet ready to assign any significance to it.

In regard to the question of a change in the mitral valve, I may say that in the same heart that exhibited the vascular changes the mitral valve was thickened, both leaflets showing many mitotic figures, which may be indicative of active proliferation, or may be normal. I do not know. I have not yet found it in the normal. Further studies may reveal more.

Dr. Jacobson inquired as to remissions. During the first month of the arthritis there is a migratory phase with both strains. The process begins in one or two joints, may then leave them and appear in others, and sometimes there may be a short period of about a week when all the joints have cleared up, and then they may come back again. With Strain A, 90 per cent of the mice seem to be rid of their arthritis after that time, whereas with Strain B it settles down in one, two, or more joints, and progresses to a condition where many of the mice are quite crippled with extensive ankylosis.

Dr. Goldberg mentioned arthritis in animals in which other bacteria were found. Whether or not the *B. coli* or streptococci were etiologically related to the lesions he saw, we all know that experimental arthritis in one form or another has been produced with various bacteria. Our interest in the particular group of microorganisms I just described is that they have specific tissue affinities. On the one hand we have a strain which inoculated into any other part of the body will do nothing. When it localizes in the joint it is capable of carrying on certain processes which lead to a chronic, progressive proliferative arthritis. Another strain has somewhat broader affinities but is still restricted to tissues of mesenchymal origin. It may be just another group of microorganisms which we have not known before producing something which is similar to certain manifestations of human disease, and may not have anything to do with the actual etiology of the human conditions, but as far as I know, the experimental syndromes I have described are quite different from anything that one has been able to produce with the known pyogenic microorganisms.

I can only thank Dr. Weller for his discussion.

TRANSMISSION OF ENDOCARDITIS LENTA TO RABBITS. Ward J. MacNeal and (by invitation), Martha Jane Spence and Marie Wasseen, New York City.

Abstract. Endocarditis has been produced by injury to the heart valves followed by infection with various bacteria in experimental animals by many investigators since the pioneer work of Rosenbach in 1878. Dreschfeld in 1887 succeeded in transmitting the disease to rabbits by simple intravenous injection of pure cultures and this was confirmed by Horder in 1907. The present authors have been able to transmit to rabbits by repeated intravenous injection of serum-broth cultures of *Streptococcus viridans* the specific type of vegetative endocarditis due to this organism and best designated at present by the name endocarditis lenta. This we regard as production of the disease by pure cultures in the bacteriological sense of Robert Koch.

Discussion

(Dr. Joseph Tannenberg, Albany, N. Y.) In rabbits which by repeated injections (6 to 9) had been immunized against horse serum or pneumococci or streptococci small petechial hemorrhages were frequently found within the tissue of the mitral valves. I would like to inquire if Dr. MacNeal has also seen such lesions in animals which perhaps were sacrificed in the early stages of his experiments. Such lesions might possibly form the local basis for the endocarditis obtained in the end-stage of these experiments.

(Dr. A. B. Wadsworth, Albany, N. Y.) Dr. Tannenberg has stressed the point which I think is crucial in this conclusion of Dr. MacNeal's: that the induction of endocarditis experimentally does not depend upon damage to the valves. Of course the injection of this material introduces an artifact and the possibility of local injury. That was quite commonly observed in studies on the development of endocarditis in immunized animals which I published some years ago. In those animals I pointed out that there was in the primary lesion a hemorrhagic extravasation, an action of the toxins on the blood vessels, as the primary predisposition to the lesion. Furthermore, these lesions occurred in the process of immunization. The streptococci, pneumococci and meningococci give rise to this experimental endocarditis in horses under immunization. The action of the streptococcus toxin is possibly very definitely dependent on a condition of tissue susceptibility, sensitization, or possibly the specific sensitization of immunization, a partial state which I reported in 1918 (*J. A. M. A.*, 1918, 71, 2052). It has come forward in the studies of the relation of the streptococcus to scarlet fever — a selective action on the blood vessels. With the introduction of this culture material, I wonder if you could exclude the possibility of the action of these toxic injections on the blood vessels being a primary injury, and predisposition to the localization of the microorganisms in the valves. That is what appears to take place in these very common endocardial lesions that we get in horses under immunization with streptococcus, with pneumococcus, and even with meningococcus.

(Dr. B. J. Clawson, Minneapolis, Minn.) I should like to ask Dr. MacNeal if he studied the myocardium.

(Dr. Otto Saphir, Chicago, Ill.) I should like to ask Dr. MacNeal why he calls this condition endocarditis lenta rather than infective endocarditis, or acute bacterial endocarditis.

(Dr. MacNeal, closing.) In regard to the early lesions, that of course we cannot answer, because we have not killed these animals a few days after inoculation, but have allowed them to develop the disease, and the animals have gone on to a natural death. So the lesions have not been early lesions. The animals have died after several days or weeks or months. I cannot answer that question; there are a great many problems arising which would require further study.

In regard to the question of local injury, it seems that is a point which perhaps can be studied, but I do not know of any exact observations which would bear on it. However, when one inoculates any microorganisms into the body, it is difficult to exclude the action of some injurious agent. The point I wish to make here is that by a simple technic, lacking in modern refinements, but similar to that used in the beginning days of bacteriology, we have been able to transfer a disease to the rabbit just by taking a serum-broth culture of the causative microbe and repeatedly injecting it into the ear vein.

In regard to the myocardium, I would say there are lesions in the myocardium. I am not in a position to make any more definite statement at this time. I should be delighted to submit some of these sections to Dr. Clawson, if he is interested.

The question as to why we chose to call the disease endocarditis lenta is raised. I believe this is important. I think we are now at a point where we should cease to talk about endocarditis as a single disease entity. We should get down to brass tacks in regard to the etiology, and I choose to use the term endocarditis lenta here because it more nearly represents the designation of a specific infectious disease due to a specific type of microorganism than any other term which is commonly employed. I do not mean here the causation of an endocarditis by some cocci or some bacilli, but I mean a specific infectious disease due to a particular type or group of streptococci, which is the common cause of this disease in man. I believe it is time for us to give a specific etiological diagnosis to lesions of heart valves, according to the specific microbic cause, just as, instead of talking about granuloma, we should distinguish between syphilis, tuberculosis, leprosy, and a foreign body reaction.

EXPERIMENTAL PNEUMOCOCCIC MENINGITIS. (*a*) PERMEABILITY OF CEREBROSPINAL BARRIER TO ANTIBODIES. (*b*) LACK OF IMMUNITY OF RECOVERED ANIMALS. Paul Gross and (by invitation) Frank B. Cooper, Pittsburgh, Penn.

Abstract. Type I horse and rabbit antipneumococcic serums were effective in experimental Type I pneumococcic meningitis in rats. This suggests that the cerebrospinal barrier is more permeable in the rat than in the dog or man. Type III rabbit antipneumococcus serum, while effective in experimental Type III pneumococcic pneumonia in rats and Type III pneumococcic sepsis in mice, was without demonstrable therapeutic action in Type III pneumococcic meningitis in rats. This ineffectiveness of the Type III rabbit antipneumococcus serum is interpreted as indicating an antibody too large to pass the cerebrospinal barrier.

Rats which recovered from pneumococcic pneumonia showed a fair degree of immunity to subsequent intraperitoneal infection with one to ten fatal doses of the homologous strain; whereas rats similarly recovered from pneumococcic meningitis showed no immunity.

Discussion

(Dr. A. B. Wadsworth, Albany, N. Y.) In my early experiments on the action of serum on pneumococcus infection in rabbits I worked with the homologous rabbit serum and I was unable to obtain any survival by the administration of serum 4 hours after the intravenous injection of pneumococci. In these experiments the inoculation is a local one. The serum was administered 6 hours later and a variable number of the animals survived. The question arises as to localization of the infection in each instance, that is, how soon the pneumococci arise to a generalized bacteremic infection. I wonder whether or not this might have been an important determining factor in the survival or death of the animals in these experiments.

(Dr. Gross.) In answer to that question, the evidence which we have is to a large extent circumstantial. We have made a study of the residual lesions in the recovered animals. This study involved a series of about 70

animals, and in every one of these recovered animals we found residual lesions. They were of variable degree and distribution. We found generalized, but not uniformly distributed thickening of the meninges. We found lymphocytic infiltration. We found various types of cortical lesions, not only over the cerebral hemispheres, but also the cerebellum. We also found less pronounced meningeal changes in the spinal cord.

(Dr. Wadsworth.) I meant generalized bacteremia.

(Dr. Gross.) Bacteremia was present in 100 per cent of the animals tested 4 hours after the infection in a previous analogous series.

THE INFLUENCE OF SULFANILAMID UPON THE EVOLUTION OF EXPERIMENTALLY-INDUCED PNEUMOCOCCUS PNEUMONIA IN RATS. David Goldstein (by invitation) and Irving Graef, New York City.

Abstract. Following the technic of Nungester and Gunn, and Mellon and his associates, we have introduced a mixture of gastric mucin and pneumococci into the bronchial trees of rats, inducing a pneumonia of lobar proportions which bears a striking resemblance to the gross and microscopic picture observed in man. Culture dilutions of 1:10 of a single Type III strain were used to provide an inflammatory pattern in 80 albino rats upon which the influence of sulfanilamid was studied.

Animals in the treated groups were given sulfanilamid (Winthrop Chemical Company) in olive oil emulsion in daily doses of 0.75 mg. per gm. of rat by subcutaneous route. The first administration of the drug was made within 2 hours of the operation and repeated once daily. Except for animals in survival experiments, the rats were divided in groups and sacrificed serially at daily intervals. Just prior to sacrifice tail blood cultures were obtained. Autopsies were performed at once under sterile conditions, and cultures of the lung were made.

The death rate among 26 controls was 96 per cent and the average time of death 3.2 days. With the exception of two late deaths (5th and 7th days) all control animals died on or before the 4th day. Nine control rats were sacrificed during the first 48 hours. Of 10 animals treated 8 days and designated for 15th day sacrifice there was a death on the 8th and another on the 11th day. Among the 37 treated animals serially sacrificed (1–7 days) there was a single 5th day death.

A comparison of the pneumonia in the treated and untreated groups demonstrates that sulfanilamid altered the course and the extent of the induced pulmonary infection. This was apparent at 24 hours, where the lesion was limited in extent of consolidation, and showed smaller amounts of edema fluid. The treated animals showed at 24 hours a great reduction in the number of bacteria present, and in some, despite a well established fibrinopurulent pneumonia, organisms could not be found either in section or by culture. The control animals all showed a spreading fibrinopurulent pneumonia with myriads of bacteria. Pleural involvement occurred in 92 per cent of the control animals, and in 23 per cent of the treated. The incidence of pulmonary infarction, thrombosis and abscess formation was approximately 25 per cent in untreated animals, slightly less in treated. Lung and blood cultures of control rats were uniformly positive for pneumococci at death. A single positive blood culture was observed in the treated group — this from one of the three fatalities. Pneumococci were recovered from 30 per cent of the lung cultures

of the treated group, and these grew on blood agar as smooth, mucoid (virulent) colonies and gave a positive Neufeld reaction.

The striking finding in the microscopic picture of treated pneumonias 2 or more days old was the predominance of the mononuclear cell in the alveolar exudate, which at 24 hours was comprised of polymorphonuclear leukocytes. This change is correlative to the findings of Robertson and his co-workers in immunized dogs recovering from experimentally induced pneumonias. The appearance of the monouclear cell is closely associated with the disappearance of bacteria; this is supported by the observations of Nungester and Gunn of the appearance of mononuclear cells and the disappearance of organisms from the lungs at 48 hours when dilute infecting doses of pneumococci were used.

Phagocytosis of bacteria was inconspicuous in all treated animals and appeared to play no significant part in the disappearance of bacteria. While no immunological studies have been made, antibody titrations in pneumococcal infections in man, dog and mouse suggest that the early disappearance of organisms in treated rats probably precedes the formation of antibodies. The mode of action of sulfanilamid would seem to be bacteriostatic and bactericidal in the light of this work.

Discussion

(Dr. Herbert L. Reichle, Cleveland, Ohio.) Before the organisms disappeared, were there any observations made on the capsule in the treated animals?

(Dr. Theodore J. Curphey, Westbury, N. Y.) I would like to ask whether cultures of the lungs of the rats at postmortem showed organisms other than the pneumococcus injected. I did some experiments along these lines not so long ago, and felt that because of the presence of so many contaminating organisms, especially in the control group, that the picture did not give a fair comparison with the lesion found in pneumococcus pneumonia in human beings.

(Dr. Goldstein, closing.) As to the demonstration of capsules, one can only gather that by inference from the refractory zone about the pneumococcus. My attempts to demonstrate capsules by capsule stains in the tissues have not been successful. I am familiar with the work demonstrating the disappearance of capsules, but we cannot make any definite statements about this.

As to a variety of organisms in the cultures, with the technic we used, pure cultures were obtained in something like 99 per cent of cultures at autopsy, and this result obtained even if the animal died during the night and the autopsy was made the next morning.

STUDIES ON THE HISTOPATHOLOGICAL CHANGES PRODUCED IN RABBITS BY EXPERIMENTAL INOCULATION WITH THE HEMOLYTIC STREPTOCOCCUS, ITS NUCLEOPROTEIN AGGLUTINOGENIC FRACTION AND 9 PER CENT SAPONIN. PRELIMINARY REPORT. Lawrence W. Smith, Isabel M. Morgan (by invitation) and Stuart Mudd, Philadelphia, Pa.

Abstract. The experimental material consisted of a series of 65 rabbits inoculated variously with whole organisms, with chemical fractions of these organisms and with another known hemolytic agent (saponin) as a control. In most of the work hemolytic streptococci of Lancefield Group A were used, but in addition, the effect of organisms of Groups B, C and D on the animals

was also studied. In general, it may be stated that no changes either of a toxic or vascular nature were demonstrated in the normal controls; that either no recognizable, or at the most only minimal changes could be found in those animals inoculated with nucleoprotein agglutinogen; that both toxic degenerative parenchymatous changes, and vascular lesions indistinguishable in kind from those reported by one of us (L. W. S.) in human fatal scarlet fever, occurred in these animals injected with whole streptococci, whether through the use of heat-killed organisms or by the infected subcutaneous blood clot method; that saponin as a hemolysin tended to produce severe degenerative parenchymatous visceral lesions; and finally, that similar toxic and vascular changes could be demonstrated in animals treated by injection of the partially purified lytic fractions. Similar changes differing only in being of lesser degree were noted in animals inoculated with the B, C and D types of hemolytic streptococci. From this material the hypothesis suggests itself that the nucleoprotein fraction might prove to be of value as an immunizing agent, in experimental infections in animals, thus avoiding the harmful effects of the toxic lytic fractions.

INTRACYSTIC PAPILLOMA OF THE BREAST. Otto Saphir, Chicago, Ill.

Abstract. A histological investigation of 58 intracystic papillomas of the breast reveals three distinct varieties of these tumors: a fibrous type, a glandular type, and a papilloma consisting of cells which closely resemble the transitional epithelium of the urinary bladder, and hence, may be designated as the transitional cell type. The fibrous type consists of a stalk of connective tissue which often is very thin. Ramifications of the stalks may fuse with the production of pseudoglandular structures. This is the most common type of papilloma. Because of such pseudoglandular structures, this special subgroup of the fibrous variety may be designated as the pseudoglandular type. The glandular type is apparently formed by an extension of neighboring hyperplastic or adenomatous periductile acini into a duct or cyst, the epithelial cells of the duct enclosing the invaginated acini. Both the fibrous and the glandular types are benign tumors which do not recur. They often extend into the neighboring ducts but cannot be regarded as precancerous. The transitional cell type of papilloma morphologically is a benign tumor. However, it may recur after removal and some of the recurrent tumors may show morphological evidence of malignancy. It resembles the papilloma of the urinary bladder which, though morphologically benign, is sometimes classified as carcinoma Grade I principally because of recurrences which prove to be malignant. Intracystic papillomas of the breast are as a rule multiple. The multiplicity of these transitional cell papillomas may be explained on the basis of multiplicity of origin or by implantations of tumor cells in neighboring ducts. Such implants do not necessarily indicate malignancy, but may be compared to certain benign ovarian tumors which occasionally produce implantations on the peritoneal surface.

Discussion

(Dr. Carl V. Weller, Ann Arbor, Mich.) I should like to ask if Dr. Saphir finds the basal cell layer of the breast concerned in any of these three types of papilloma.

(Dr. Saphir.) It is usually the upper layers of the cells lining the duct structures, rather than the deeper layer, which are concerned in papilloma.

SPLENIC NEOPLASMS. Samuel A. Goldberg, Newark, N. J.

Abstract. Primary neoplasms of the spleen are quite rare. A review of the literature shows mostly reports of individual cases. The various types of primary neoplasms reported in order of their occurrence are: lymphoblastoma, lymphangioma, hemangioma, endothelioma, and rarely fibroma and fibrosarcoma. Some of the cases of lymphosarcoma reported in the earlier works, according to Klemperer, should be discounted as they probably belong in the group of lymphadenoses or atypical Hodgkin's disease.

The angiomas range from simple localized or widely diffuse telangiectases to more or less encapsulated nodules, and finally to highly malignant endotheliomatous tumors with metastases.

Metastatic neoplasms of the spleen are also seldom encountered. This is not in keeping with the nature of the organ since embolic phenomena are fairly common in the spleen. As the spleen is both a vascular and a lymphatic organ, metastatic neoplasms should be more commonly seen. The paucity of such metastases cannot be explained on an entirely morphological basis, such as the limitation of lymphatics to the subcapsular region, the sharp angle of origin of the splenic artery, or the effect of splenic pulsation in preventing the lodgement of tumor cells. The explanation lies more likely in the fact that the spleen offers a poor soil for the growth of neoplastic cells. Lubarsch first pointed out that in order for cells to establish themselves in an organ they must overcome the resistance of the organ, and several generations of cells are destroyed before they can adapt themselves and are able to proliferate. Fichera speaks of an oncological ferment which hinders the proliferation of tumor cells. Krumbhaar states that the occurrence of fibrous nodules in the spleens of cancer patients may be further evidence of the antagonism of splenic tissue to malignant tumors. McNee reported 2 cases with development of epithelial tumors several years after an enlarged spleen had been removed. He ascribed the formation of these tumors to the removal of the factor in the spleen which hindered the growth of epithelial neoplasms.

Experimentally several investigators have found that splenic pulp mixed with transplanted tumor cells inhibited or retarded the growth of the tumors.

This report includes 3 primary and 9 secondary neoplasms of the spleen collected from 540 autopsies, 93 of which showed malignant tumors. This gives an incidence of 9.7 per cent of secondary malignant splenic neoplasms. Of this number, 5.37 per cent were gross nodular tumors, 2.2 per cent were microscopic metastases, and 2.2 per cent showed invasion by contiguity into the splenic pulp.

The neoplasms primary in the spleen were 1 hemangioma cavernosum and 2 lymphangiomas.

Discussion

(Dr. E. T. Bell, Minneapolis, Minn.) I want to comment on the statement that the spleen is an unfavorable site for the growth of metastatic tumors. Someone about a year or so ago showed that the kidney has metastases less frequently than the spleen. The combined weight of the kidneys is a great deal more than the weight of the spleen, and yet they have fewer metastases

than the spleen. Our own autopsy records show the same thing. It is also true that the heart has metastases less frequently than the spleen, so if the spleen has some anticarcinogenic substance in it, the kidneys should have more.

(Dr. Howard T. Karsner, Cleveland, Ohio.) I am in full agreement with what Dr. Bell says. I think the paper justifies some comment on the use of the term neoplasm. To my mind the inclusion of the so-called hemangioma and lymphangioma, or mixtures of these, as true neoplasms, appears to be unjustified. This view naturally involves consideration of the place of the hamartomas.

(Dr. Kornel Terplan, Buffalo, N. Y.) I should like to stress the occurrence of the microscopic type of metastatic carcinoma in the spleen. I remember a few instances in our postmortem material of the last years in which the spleen was of normal size or even slightly atrophic, with no gross evidence of metastatic tumor. The site of the primary carcinoma was the stomach or the lung. Microscopically the spleen was found to be studded with carcinoma cells; they did not form nodules but had grown diffusely throughout the sinusoids. In 1 of these cases the liver showed a similar type of metastatic carcinomatosis. It was small and anemic. There was no gross evidence of carcinomatous nodules. Histologically, however, the capillary sinusoids showed most diffuse infiltration with carcinoma cells. From such surprising experiences I feel that microscopic metatasis to the spleen is apparently more frequent than we were inclined to expect.

(Dr. Goldberg, closing.) In regard to Dr. Karsner's remark about the question whether lymphangiomatous or hemangiomatous tumors are really neoplasms, according to the classification of Fowler they are supposed to be neoplastic lesions. The fact that they are nodular and have a stroma, according to the literature, is an indication that they are neoplastic. Some of these lesions may be simply telangiectatic, as I pointed out.

The fact that in 93 cases of malignant neoplasms there were 9 cases of metastases in the spleen certainly indicates that they are not as rare as the literature would have us think.

CARCINOMA OF THE LUNG. AN ANALYSIS OF SEVENTY-FOUR AUTOPSIES. Rigney D'Aunoy, Bjarne Pearson (by invitation) and Béla Halpert, New Orleans, La.

Abstract. Seventy-four cases of primary carcinoma of the lung were encountered in 6623 autopsies on individuals over 1 year of age. Males and females were represented in the proportion 11:1. The age range was from 21 to 75 years. The average duration of illness was 5 months. Thirteen patients died in the 5th, 33 in the 6th, and 19 in the 7th decade of life.

In almost half of the cases the primary growth was located in one bronchus or the other.

Thirty-seven of the 74 cases were squamous cell, 21 were reserve cell, and 16 were columnar cell carcinoma.

Discussion

(Dr. Emmerich von Haam, Columbus, Ohio.) I should like to ask if there is a difference in the distribution of metastases in the 3 carcinomas.

(Dr. H. Gideon Wells, Chicago, Ill.) How large a proportion of this

great incidence of carcinoma of the lung may be attributed to the greater zeal in securing autopsies in cases of this class because the diagnostic problem excites interest on the part of the clinicians? It seems to me that has modified our statistics a great deal in institutions where there is not 100 per cent of autopsies, and I do not know of any such institutions.

(Dr. Howard T. Karsner, Cleveland, Ohio.) In another large city hospital the number of bronchogenic carcinomas disclosed at autopsy exceeded that of any other form of carcinoma, and I think we should refer to figures of this sort as representing the autopsy room population rather than the living population.

(Dr. James Ewing, New York City.) I should like to know if the authors found any evidence of incidence of the relatively benign papillary adenocarcinoma of the bronchi described by Crawford of Philadelphia. I would also like to ask if they draw any conclusions as to whether there are tumors derived from the lining cells of the alveoli.

(Dr. Wiley D. Forbus, Durham, N. C.) There is an interesting bronchial tumor which Dr. Halpert has not mentioned. It is one which has the gross characteristics of a colloid carcinoma. I use the term carcinoma with some hesitation because the tumor is one which does not invade very widely but acts rather like a benign tumor. Histologically, however, it is definitely invasive. It would be interesting to know where Dr. Halpert puts this tumor in his classification.

(Dr. Harry C. Schmeisser, Memphis, Tenn.) Drs. W. Likely Simpson and Robert M. Moore, from our departments of otolaryngology and pathology, published a case of primary colloid adenocarcinoma of the lower third of the trachea, which was shown to take origin from the epithelium of the mucous glands below the surface.

(Dr. Halpert, closing.) In reply to Dr. von Haam's question, there is no significant difference in the metastases of one type or the other type of carcinoma. This series has been too small to draw any conclusions in that direction.

In reply to Dr. Wells, of course this represents autopsy material. However, in this particular series perhaps it does not represent increased zeal in securing autopsies. These were patients who quite frequently died without a correct diagnosis, and the finding at autopsy of a carcinoma of the lung was accidental.

In answer to Dr. Karsner's remark, perhaps his hospital deals with the same kind of clientele as ours, and that may be the reason that he can put one over on the Charity Hospital as far as the number of autopsies of carcinoma of the lung is concerned.

As to Dr. Ewing's question, this is a study of 74 carcinomas seen at autopsy, and since I have not come across the kind he mentioned, I could not say anything about it. In reply to his question as to whether we found evidence of origination of carcinoma from the alveolar lining cells, I have not found any of those. I feel quite confident that the lining cells of the alveoli of the lung rarely, if ever, give rise to malignant neoplasms. The parent cells of all carcinomas of the lung, I believe, are the reserve cells.

In regard to the comments of Dr. Forbus and Dr. Schmeisser, I would regard both of those tumors as columnar cell carcinomas, and they of course can produce mucus. They are derived from reserve cells which differentiate into the cell form which they normally produce.

STROMAL TUMORS OF THE CHOROID PLEXUS. Amour F. Liber and James R. Lisa, New York City.

Abstract. The choroid plexus is made up of two kinds of tissues, the epithelium and the stroma, which are distinct embryologically and anatomically. The stroma is an infolding of the leptomeninx. Tumors derived from the stroma are indeed homologues of those of the meninges. Meningioma is the most common neoplasm of the stroma. Others are sarcoma, angioma, chondroma, lipoma, myxoma, teratoma and deposits of calcium, iron or cholesterol with phagocytic and fibrotic reactions. In man, meningioma, sarcoma and angioma are the only neoplasms that reach a large enough size to be of clinical importance.

A typical case of meningioma of the choroid plexus of the left lateral ventricle has been studied. The tumor filled and distended the entire body and parts of the frontal and occipital horns of the ventricle. It was attached to the dorsal aspect of the glomus by a short stalk of fibrous tissue and blood vessels covered by choroid tufts. Microscopically elongated bundles of syncytial bands containing fibroglia and fusiform vesiculous nuclei were lined up end to end in long straight rows. Collagenous and reticulum fibers coursed between the cytoplasmic bands. In some places fine reticulum fibers came into close contact with the cells and seemed to penetrate them. No elastic fibers were found. Occasionally there were cellular whorls which contained no collagen and only rare reticulum fibers. No mitoses were seen. Blood vessels were scarce and thin walled. There were no calcospherites. The tumor was surrounded by a thin hyalinized capsule, which was partially adherent to the ventricular wall. The ependyma persisted in some of the adherent areas but was absent in others.

The remaining cases were autopsy discoveries. Two cases revealed large, cystic calcareous deposits surrounded by dense fibrotic scar tissue. A small fibrolipoma was found in the plexus of the lateral ventricle. The fibroblastic portion of the growth was sharply demarcated from the adipose tissue and presented the features seen in fibrosed arachnoidal granulations. Adipose tissue is not a normal constituent of the choroid plexus stroma. It is a question whether it arose as a heteroplasia or as a transformation *in situ* of leptomeningeal tissue. Reports of only 4 previous cases of lipoma of the choroid plexus could be found. In 3 cases of congenital hydrocephalus with spina bifida in infants, narrow fibrovascular stalks projected dorsalward from the glomus of the choroid plexus bilaterally. The tip of the stalk was thickened to form a terminal nodule consisting of hyperplastic cellular and collagenous stroma which, similar to that seen in fibrolipoma, had a structure suggestive of arachnoidal granulations. In 1 case calcospherites were present. In a case of unilateral hydrocephalus, associated with an anomalous arrangement of the large veins of the ipsilateral cerebral hemisphere, a long stalk projected dorsalward from the glomus in the dilated ventricle only. There was no terminal nodule.

In all of these cases the growth occurred in or was attached to the glomus of the choroid plexus. The hypothesis is formulated that hydrocephalus, the formation of a stalk and the hyperplastic nodules are steps in the development of meningiomas. In this respect, as in their structure, the hyperplastic glomic nodules would seem to be analogous to the arachnoidal granulations.

Discussion

(Dr. Ralph D. Lillie, Washington, D. C.) In regard to the possible histogenesis of this fibrolipoma, if you call it that, of the choroid plexus, I might remark that fat is not too infrequently a component of the stroma of the choroid plexus in some of the laboratory animals I have been studying in recent years.

(Dr. Liber.) Some varieties of lipid have been reported in a number of animals. I am familiar with that in horses. It has been found by veterinarians that 8 per cent of old horses (I do not know what age they call old in a horse) have xanthomas, sometimes called cholesteatoma vasculosa. From the description given by different pathologists who have studied these growths, they are not lipomas but xanthomas with doubly refractile lipids and cholesterol crystals, foreign body giant cells, and as a rule an intense inflammatory reaction. This is in the horse.

(Dr. Lillie.) That is not the type of thing I am referring to. It is apparently ordinary body fat.

(Dr. Liber.) In what animals?

(Dr. Lillie.) In guinea pigs in particular.

METASTATIC TUMORS OF THE MYOCARDIUM. Gorton Ritchie, Madison, Wis.

Abstract. Fifteen cases of tumor metastases in the myocardium have been studied. Thirteen different types of primary tumor were found. Carcinoma of the lung occurred 3 times; there was 1 each of the following tumors: rhabdomyosarcoma of the kidney, carcinoma of the esophagus, mesothelioma of the pleura, myogenic sarcoma of the bladder, melanosarcoma, carcinoma of the rectum, carcinoma of the fundus uteri, carcinoma of the head of the pancreas, and lymphosarcoma (primary source undetermined). The malignant cells were carried to the heart through the blood stream in a majority of the cases, but the spread in the cardiac wall was frequently by way of the lymphatics. In 1 case (carcinoma of the esophagus) the only "remote" metastasis was to the heart muscle by way of the blood stream; in all of the others the metastases were fairly widely distributed (except 1 case of sarcoma of the lung in which the invasion of the heart was by direct extension). Although involvement of the heart was quite extensive in some cases, in no instance was the diagnosis made clinically.

In addition, 23 metastatic tumors of the pericardium were found, representing 11 different types of primary tumor.

Discussion

(Dr. William Boyd, Toronto, Canada.) May I ask Dr. Ritchie where the metastases were in the case which he showed as being one of sarcoma? The evidence which he adduced in support of that diagnosis was that the tumor was massive in character and that the reticulum stain suggested sarcoma. Certainly a massive growth in no way eliminates carcinoma. I think Dr. Karsner will agree with me when I say that one has to be very careful in drawing conclusions from the use of the reticulum stain. I feel in these cases the natural history of the disease, in other words the distribution and character of the metastases, is of the greatest value in deciding between carcinoma and sarcoma, and I think the speaker would have to give us very convincing

evidence to make us accept this tumor as a sarcoma, judging from the fleeting glance I had of it on the screen.

(Dr. Carl V. Weller, Ann Arbor, Mich.) Within the last 3 weeks we have had a case similar to the one reported, a squamous cell carcinoma of the esophagus with metastases to the apical portion of the left ventricle without pericardial adhesions and without any traceable extension by contiguity in the lymphatics.

(Dr. Ritchie, closing.) I was almost afraid to include this lantern slide for fear it would lead to such a controversy. The metastases were merely local in this case and were not widespread throughout the body. It is on the evidence of the definite small spindle cells within the tumor and the very apparent connection of the reticulum with these cells that I have proffered this diagnosis.

CONCEPTS OF A NEW CLASSIFICATION OF OVARIAN TUMORS. Walter Schiller (by invitation), Chicago, Ill.

Abstract. Several attempts have been made during the course of the last few years to suggest a better and more satisfactory classification of ovarian tumors than that usually found in the textbooks of pathology or gynecology. Counseller and Broders, Cornill, and Leroux, Levret and Weinroth suggested classifications more or less founded on anatomical or clinical facts. These classifications serve very well for practical and clinical purposes, but do not satisfy the aim of the pathologist to have a system founded on the histogenetic explanation of the various newgrowths. Recent experiences and investigations justify trial of a histogenetic system. First the cysts which are physiological cavities dilated by pathological secretion or retention are separated from the true neoplasms. These cysts are classified according to the cavities from which they develop as follicular cysts arising from follicles, corpus luteum cysts arising from corpus luteum, and corpus atreticum cysts usually called lutein cysts. The neoplasms may be split into two large groups — those developing from true ovarian tissue or ovariogenetic, and those developing from tissue which is not found in the normal ovary, or heterotopic tumors. The ovariogenetic tumors include the fibroma from the fibrous tissue and the granulosa cell tumor from the granulosa. The heterotopic tumors have as the first subgroup tumors developing by protoplastic differentiation from the surface of the epithelium. They duplicate either tubular epithelium and form serous cystomas, or the endometrium, forming endometriomas, or the cervical mucous membrane, forming pseudomucinous cystomas. The second group is represented by error in sex chromosomes occurring in fetal remnants of the mesenchyma. When the sex differentiation is male they form arrhenoblastomas; when the differentiation is neither male nor female, but neutral, they form disgerminomas. The third group contains the tumors developing from misplaced blastomeres. This group includes the mature teratoma represented by the dermoid and the immature teratoma or embryoma. The fourth group develops from misplaced neighboring tissue and is represented by hypernephroma, ganglioneuroma, Brenner tumor (epithelium of uropoietic organ), and mesonephroma. The fifth group contains tumors developing by an actual transference of extraovarian tissue to the ovary during life. This group can be divided into two subgroups: the metastatic tumors (for instance, the Krukenberg tumor) and those particular endometriomas

originating by implantation of endometrium on the surface of the ovary, according to Sampson.

Discussion

(Dr. James Ewing, New York City.) Dr. Schiller proposes to wipe the slate clean and start over again classifying ovarian tumors on a histogenetic basis. Such an effort is to be commended, if it can be made successful. The majority of ovarian tumors are cystadenomas which are readily identified, although their exact histogenesis may still be somewhat uncertain. The difficulty arises when dealing with a smaller group of malignant tumors of which the structure varies extremely and the histogenesis is highly obscure. In this latter group most pathologists recognize some types which are rather specific and easily recognized, such as the Brenner tumor, the seminoma, and malignant variants of the dermoid, but most of these cellular tumors present a very varied and often mixed structure which it has been difficult to interpret. In recent years the studies of Robert Meyer and many others have brought new data into this field by showing that striking changes in the secondary sex characteristics occur in certain of these tumors, and they have undertaken the classification of these growths in the light of these hormonal effects. New interpretations of the embryology of the ovary have also been introduced as guides in the classification. Varangot has published recently a very excellent monograph covering all these new data and has presented a classification based thereon. When one reads the new contributions one may obtain the impression that it is now comparatively easy to identify the cellular malignant tumors, especially when clinical data relating to the sex changes and the endometrium are available. However, after arming myself with much of this new information I found to my great disappointment that the atypical malignant tumors were about as difficult to recognize as ever. The structure varies extremely, the sex changes are not reliable, and the embryology of the ovary is highly obscure. Moulonguet has said that the embryology of the ovary offers no reliable basis for the classification of ovarian tumors. The subject of intersexuality proves to be even more complex than that of ovarian tumors. Recently, in reviewing a series of complex malignant ovarian tumors and observing the diagnoses submitted by pathologists who have enjoyed some reputation in this field, I came to the conclusion that the final diagnosis adopted is largely a matter of arbitrary decision on the part of the observer. No doubt many of the so-called granulosa cell tumors with feminizing properties are recognizable with certainty, but many others are not. Arrhenoma presents a very mixed and variable structure of spindle and polyhedral cells, and masculinizing effects are not limited to tumors derived from male elements. Theca cell tumors and so-called luteinizing forms of granulosa cell tumors must be identified on very uncertain grounds, such as fat droplets. The specific structures assigned to these various tumors by many writers obviously overlap one another.

Under such circumstances I cannot think that adequate knowledge is yet available on which to establish the actual nature of many malignant ovarian tumors. Dr. Schiller's feeling that we must revert mainly to a morphological basis and endeavor to establish the histogenesis of these tumors seems therefore to have considerable support, but I fear that here again the difficulties are quite formidable. I can report the observation of 1 minute ovarian carcinoma, 3 mm. in diameter, submitted recently by a Brooklyn physician.

Unfortunately it had a very mixed structure, some acini resembling Pick's testicular adenoma, others suggesting a granulosa cell tumor, with a few strands of spindle cells like arrhenoma.

(Dr. Harry C. Schmeisser, Memphis, Tenn.) I recently published in collaboration with Dr. W. A. D. Anderson the report of a ganglioneuroma of the ovary which could easily be diagnosed from its histology. We considered its origin to be from the groups of sympathetic ganglion cells which occur normally in the medulla of the ovary near the hilus. I wish to ask Dr. Schiller where he would place this tumor in his classification.

(Dr. Schiller, closing.) The ganglioneuroma falls into the group of tumors developing from neighboring tissue misplaced in the later period of fetal life.

Concerning the papers of the French authors, especially Varangot, I have studied these papers but have the feeling that some of these men make the mistake of relying too much on physiology and too little on morphology. Whenever it is possible we should rely on the morphological, and if possible on the histogenetic-embryological changes. When one starts to classify tumors according to the products they furnish, one may have to place heterogenetic elements in one group. There would be a group called virilizing tumors, which would include hypernephroma and arrhenoblastoma, although they are morphologically and histogenetically different.

In no other field of pathology do we make our classification according to the physiological effect of the tissue, for every physiologist knows that different tissues may produce the same secretions. Whenever it is possible to furnish a classification, it is preferable to make a tentative classification on a morphological basis rather than to accept physiological classifications which may include errors.

CARCINOMA OF THE KIDNEY IN A COLONY OF RHESUS MONKEYS. Herbert L. Ratcliffe, Philadelphia, Pa. (Presented by Dr. Herbert Fox.)

Abstract. Because of the relative infrequency of neoplasms in infrahuman primates, it may be of interest to record the occurrence of carcinoma of the kidney in 4 rhesus monkeys (*Macaca mulatta*). Of further interest is the fact that these animals were members of a family group, the male parent and 3 offspring, a female and 2 males, being involved.

In each case the tumor developed in the left kidney, expanding the capsule and replacing the parenchyma. In only 1 animal were secondary tumors found.

The average age of these animals was 181.5 months, approximately 10 times the average of the group.

Discussion

(Dr. Otto Saphir, Chicago, Ill.) I should like to ask whether or not some of the tumors were bright yellow and contained fat, in other words did some of these tumors resemble the hypernephroma type of carcinoma or not?

(Dr. Fox.) Tumors Nos. 1 and 4 had a moderate number of so-called foamy cells; they were not examined for double refractile lipid bodies. Tumor No. 3 was normally opaque. It did not contain foamy cells.

THE PATHOLOGY OF MAMMARY CARCINOMA OF THE RABBIT. H. S. N. GREENE, Princeton, N. J.

Abstract. One phase of an extensive constitutional study under progress in our laboratory has been an investigation of neoplasia in a large colony of rabbits, and as a result of routine clinical and pathological examination a considerable number of spontaneous tumors have been found. Two distinct morphological types of mammary carcinoma which differ in mode of development and biological characteristics have been observed, and our type is further distinguished by a characteristic antecedent breast history. A history of cystic disease preceded the development of tumors in 21 cases, while in 4 cases no abnormal antecedent changes in the breast were noted. Tumors in cystic breasts arose as papillomas from the epithelial lining of dilated duct and cyst walls and with continued growth formed multiple radicles which anastomosed with the production of numerous acinar-like structures. In such cases cystic disease, non-invasive neoplasia and cancer occurred as succeeding events in the breast and apparently formed parts of a continuous disease process. The second class of tumors, on the other hand, originated in normal breasts and arose from a proliferation of true acini.

Pronounced pathological changes were found in organs of the endocrine system in all tumor-bearing animals and were present from the earliest stages of tumor development. On the contrary, such changes were not found in animals bearing the transplanted tumors, although more than 150 were examined after periods of growth ranging up to 6 months. Histologically the alterations were identical with those observed in animals subjected to long continued treatment with oestrone, and because of this it is suggested that the spontaneous tumors represent a natural analogue to the experimental induction of neoplasia with such substances.

Discussion

(Dr. Arthur W. Wright, Albany, N. Y.) I am very much interested in the colony of rabbits Dr. Greene has described. We have in our laboratory a strain of rats in which mammary tumors are appearing fairly abundantly. In all cases but one they have been benign fibroadenomas. The exception was a metastasizing adenocarcinoma. In these animals we have found changes in the pituitary and adrenal glands. The latter organs have not yet been thoroughly studied but the pituitaries were increased in size and often showed small adenomas which were primarily of the chromophobe type. In general the chromophilic cells were diminished in number, particularly the eosinophils. We have been slowly inbreeding these animals and have been attempting to obtain high tumor lines. At the moment the work has not gone far enough to know whether we shall be successful in this attempt. I should like to ask Dr. Greene the incidence of spontaneous mammary tumors in his colony so far.

(Dr. Emmerich von Haam, Columbus, Ohio.) I should like to ask Dr. Greene if he has made any determination of the oestrin content in the tissues of these animals.

(Dr. Clarence M. Lightner, New York City.) Did any of the tumors occur in males?

(Dr. Greene, closing.) In answer to the last question, all the tumors were

in females. There were 2 males in the tumor line, however, which showed adenomyosarcoma of the kidney.

We have no funds or facilities for determining the oestrin content in the tissues, I am sorry to say.

Inasmuch as the tumors occur in family lines and not in the general population, an analysis of the incidence based on the colony as a whole would be without significance and has not been attempted. Relatively few animals of the line have been held to a tumor age because of inadequate facilities. At the present time approximately 100 have been under observation for 3 or more years and of these 25 developed tumors.

THE SKINS OF GUINEA PIGS AFTER FIVE YEARS OF DAILY APPLICATIONS OF 1:2:5:6 DIBENZANTHRACENE. Roland S. Aronson (by invitation), Philadelphia, Pa.

Abstract not received.

Discussion

(Dr. Hugh G. Grady, Cambridge, Mass.) May I ask Dr. Aronson if he has attempted any subcutaneous injections of the carcinogenic agent in guinea pigs? I ask that question because Dr. Shear in collaboration with Drs. Elliot and Howe has succeeded in inducing subcutaneous sarcoma in the guinea pig by implantation of benzpyrene crystals.

(Dr. Stanley P. Reimann, Philadelphia, Pa.) I have no remarks to make except that the dibenzanthracene which was used was carcinogenic in mice because we used material from the same bottle and it induced carcinoma in them.

(Dr. Aronson, closing.) My answer to Dr. Grady's question is no.

HISTOLOGICAL STUDY OF THE DEVELOPMENT OF PULMONARY TUMORS INDUCED BY 1:2:5:6–DIBENZANTHRACENE AND METHYLCHOLANTHRENE IN STRAIN A MICE. Hugh G. Grady (by invitation) and Harold L. Stewart, Cambridge, Mass.

Abstract. Based on the observation that tumors can be induced in the lungs of mice treated with dibenzanthracene or methylcholanthrene, an experiment was devised whereby a study could be made of serial histological sections of tissue from the lungs of mice in the period during which the pulmonary tumors were developing.

Two hundred Strain A mice, 2½ to 3 months of age and equally divided as to sex, were used. One hundred mice received 0.8 mg. of 1:2:5:6-dibenzanthracene in 0.8 cc. of lard subcutaneously; 60 mice received 1.6 mg. of methylcholanthrene in 0.4 cc. of lard subcutaneously; and 40 mice received 0.8 cc. of plain lard subcutaneously and served as controls. Animals which developed tumors at the site of injection, or which died as a result of hemorrhage, infection, or any other cause, were not included in the study. The final effective number consisted of 130 mice injected with the carcinogens and 30 control animals. The animals were sacrificed daily (except on Sundays and holidays) over a period of 3 months, 98 being killed between the 26th and 60th days. In all animals the lungs were fixed per tracheam in Zenker's fluid, and the entire right lower lobe was sectioned in series. The remaining

lung tissue was embedded in paraffin and kept in reserve. Sections were routinely stained with eosin-methylene blue and Masson's trichrome technic. Foot's method for reticulum and phosphotungstic acid hematoxylin were also used on occasion.

The earliest recognizable pulmonary tumor was found 32 days after injection of methylcholanthrene and 36 days after injection of 1:2:5:6-dibenzanthracene. No difference in the character of the tumors produced by the two hydrocarbons was observed. From the 40th day onward, tumors were found with increasing frequency. All tumors examined appeared to be adenomatous growths and were histologically similar to those previously described as induced tumors; they also resembled closely the spontaneous lung tumors of mice. Few, if any, were connected with the bronchial epithelium at any point. In most instances the tumors were of multicentric origin and located close to the pleura or in actual contact with it. Immediately preceding the development of tumors and accompanying the early stages of their formation was a notable proliferation of large mononuclear cells from the alveolar walls. These cells at first varied in shape, depending apparently on their relation to the alveolar walls. Frequently they would form columns of varying length partly or completely lining the alveolar space, or they might coalesce to form small groups. These groups might project into the alveolar lumen or occur within or on the septal wall. The adenomatous nodules appeared to develop through a combination of these processes, but when developed they were remarkably uniform in appearance. The recognizable tumor was composed of more or less closely packed columns of cuboidal or low columnar cells with relatively large nuclei. The nuclei were either deeply staining or vesicular and few mitotic figures were seen. The cytoplasm was usually slightly acidophilic and finely granular and occasionally contained small particles of phagocytosed material.

Discussion

(Dr. H. Gideon Wells, Chicago, Ill.) It is interesting to me to see these early changes in induced tumors because in looking over many hundreds of spontaneous tumors among Dr. Slye's mice, all these pictures are there, and we have been impressed with the apparent evidence that these tumors in mice represent, as near as we can tell from looking at them, an epithelial tumor arising in the alveolar lining. I have seen tumors spontaneously arising in which I thought the tumor came from the bronchus. I was interested in Dr. Grady's statement, but he did not make it complete, on the character of the changes that take place in the transplants, and the thing which has impressed us is this: when these tumors produce metastases, as they often do, many of the metastases look like sarcomas, although the primary tumors do not look like sarcoma in most cases. The lung would be filled up with what looked like an epithelial growth and the metastases looked exactly like a sarcoma.

(Dr. Grady, closing.) That is an interesting question which makes another paper in itself. We have now seen close to 50 of these tumors which have been carried in serial transplants in which, along about the 3rd or 4th transplant, the tumor changes from what is apparently a typical epithelial growth to one which I do not think any one would quarrel with as being a spindle cell sarcoma. It is a very annoying and difficult phenomenon to explain. Incidentally Andervont has reported that finding. It should be noted that while most of the transplants are in induced tumors, Andervont has recently

observed this sarcomatous change in the transplant of spontaneous tumors which is of significance. That is why we hesitate to call these growths purely epithelial, even though they certainly look like it.

THE EFFECT OF THYROID FEEDING ON TUMOR GROWTH IN THYROPARATHYROIDECTOMIZED RATS. R. L. Ferguson, R. D. Templeton and Mary C. Patras (by invitation), and F. A. McJunkin, Chicago, Ill.

Abstract. In this experiment 135 thyroparathyroidectomized albino rats were used. Litter mates of the same sex and as near the same weight as possible were divided into two groups. Group 1 received Fox Chow only. Group 2 received Fox Chow which contained 0.02 per cent desiccated thyroid. The animals were kept on their respective diets for 150 days, at which time two small pieces of tumor tissue (rat spindle cell sarcoma) were placed beneath the skin through a dorsal incision at the level of the first lumbar vertebra.

The animals were weighed weekly and tumor measurements were obtained at that time. The thyroid-fed animals lived on an average of 39 days, while the animals on Fox Chow lived only 35 days.

Desiccated thyroid was observed to have a favorable effect on tumor growth. The importance of this observation, however, is diminished when one considers the stimulating effect of thyroid on the host.

Discussion

(Dr. Harry S. N. Greene, Princeton, N. J.) I should like to ask if these observations were limited to young animals. I ask this because in our experiments the action of thyroxin appears to be related to the age of the tumor-bearing animal. The administration of thyroxin to young rabbits bearing a transplanted uterine tumor results in rapid growth. In mature animals, on the other hand, this procedure results first in slow growth in a histologically more highly differentiated pattern, and finally in regression.

(Dr. Ferguson, closing.) For thyroparathyroidectomy we used young animals which were litter mates of about the same age and about the same weight. We obtained no regression; the animals died around 36 to 39 days after tumor transplants. The sex made a little difference. The male animals were slightly heavier than the females.

THE EFFECT OF COMBINED ANDROGEN AND ESTROGEN ON THE PROSTATE. Robert A. Moore and (by invitation) Allister McLellan, New York City.

Abstract. In a previous study it has been shown that the injection of estrogens in men induces certain characteristic histological changes in the prostate. These changes are essentially an exaggeration of the metaplasia and lymphoid hyperplasia found in all cases of benign hypertrophy.

In animals the simultaneous injection of androgens with estrogens neutralizes the effects of the estrogens. In women the ratio to give complete neutralization is about 1 unit weight of estrogen and 50 units weight of androgen.

A histological study of the prostate from men who received varying combinations of estradiol benzoate and testosterone propionate shows that the neutralizing ratio is also about 1:50. From this it is concluded that the tissues of the two sexes are not selectively sensitive to the homologous sex hormone.

MALABSORPTION OF FAT (INTESTINAL LIPODYSTROPHY OF WHIPPLE). H. L. Reinhart and (by invitation) S. J. Wilson, Columbus, Ohio.

Abstract. In 1907 Whipple suggested the term "intestinal lipodystrophy" for a hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal and mesenteric lymphatic tissues. In 1923 Blumgart reported 3 somewhat similar cases as "malabsorption of fat," and in 1936 Jarcho described a 5th similar case as "steatorrhea with unusual intestinal lesions" and reviewed the previous cases. A 6th case presenting the characteristic anatomical features and a clinical picture similar to that described by Whipple and Jarcho as anemia, progressive emaciation, chylous ascites, deposits of fat in the small intestines, mesenteric and retroperitoneal lymph nodes, and a relatively normal pancreas is described. In addition the patient presented the blood picture of a benign lymphocytosis suggestive of an atypical lymphoid leukemia. Although intestinal lipodystrophy is related to the xanthomatous diseases and steatorrhea in the anatomical and clinical manifestations of a disturbed lipid metabolism, it is fundamentally different from each of these conditions. The term intestinal lipodystrophy offers less objections and has more points in its favor than others suggested, particularly until more is known concerning the cause and genesis of the lesions.

Discussion

(Dr. J. D. Kirshbaum, Chicago, Ill.) Was the bone marrow studied in this case, and was the diagnosis of aleukemic lymphadenosis considered here, in view of the large liver and spleen?

(Dr. William L. Robinson, Toronto, Canada.) Some years ago I had an opportunity to study the glands from a case in which the thoracic duct was tied off for experimental purposes. This produced a tremendous enlargement of the glands with dilatation of the sinusoids of the lymph spaces, but it showed no picture such as we have seen here — just simple dilatation.

(Dr. Ellis Kellert, Schenectady, N. Y.) Did you find inflammatory changes at the root of the mesentery which might explain the obstruction? Some years ago in a similar case we encountered a definite old inflammatory mass in the mesentery, and the pictures here recalled that case to me, for the intestinal changes are identical. I believe that recently there have been 1 or 2 cases reported under the diagnosis of chyladenectasis mesenterica. There may be other cases listed under that name.

(Dr. Henry W. Ferris, Ithaca, N. Y.) Some months ago I did an autopsy on an individual in whom there were lesions somewhat similar to those reported here. The mesenteric lymph nodes were yellow and almost completely replaced by fatty tissue, and fat was found in the mucosa of the intestine. In addition there was a widespread inflammatory reaction on all the serous surfaces, which might be called a polyserositis; it was on the pericardium, the peritoneum and the pleura, and there was evidence of organization of old fibrinous deposits. That might bear some relation to the fatty deposits mentioned here. I wonder whether anything has been found in the literature which would show an association between these conditions.

(Dr. Goodman, Rhode Island.) Will Dr. Reinhart describe the findings in the spleen and liver?

(Dr. Alfred Plaut, New York City.) I would like to know whether the lymph nodes were distinctly yellowish or brownish. Only the other day while

going over the organs of a case of multiple myeloma I was struck by the brownish color of one parapancreatic lymph node. This lymph node gave the histological picture just shown by Dr. Reinhart. In addition, small foci of fatty material partly surrounded by giant cells were found in the spleen.

(Dr. Reinhart, closing.) In answer to Dr. Kirshbaum's question, no study of the bone marrow was made. The hematological study of the case was conducted by Drs. Doan and Wiseman, and a biopsy of a superficial lymph node revealed no evidence on which to base a definite diagnosis of aleukemic leukemia. Benign leukemic lymphocytosis was considered.

In compliance with Dr. Goodman's request, I wish to say that the spleen was enlarged and there was present passive congestion with multiple infarcts. There was no evidence of focal lipoid deposits in the spleen of the type encountered in some of the xanthomatous diseases. The liver was enlarged and there was a diffuse fine granularity of the surface; there was a moderate proliferation of the bile ducts, a moderate amount of portal fibrosis, and a rather marked lymphocytic infiltration of portal distribution which is encountered in portal cirrhosis. There was very little residual evidence of any preceding fatty metamorphosis in the liver.

The autopsy described by Dr. Ferris seems to have presented a similar if not an identical picture. The tissue reactions are very complicated and numerous factors undoubtedly play a part in their genesis. There appears to be some evidence that this condition is related to the leukemias, particularly monocytic leukemia, and possibly lymphoid leukemia and reticuloendotheliosis.

In answer to Dr. Plaut, the color of the lymph nodes was predominantly of a creamy rather than a brown color. There were small foci of a deeper yellow color which were more nearly orange than brown. In addition there were foci of recent hemorrhage.

In regard to Dr. Kellert's question, there were no inflammatory nodes at the root of the mesentery; the nodes at the root of the mesentery and around the pancreas were similar in character to the retroperitoneal lymph nodes. The retroperitoneal nodes were larger than those in the mesentery and this was the only difference noted. Certainly stasis of this lipid material is present, and it probably is a functional factor, but evidence of a primary blockage of the lymph channels was not obtained. I might say that numerous cases have been reported in the literature in which blockage of the lymph channels has been demonstrated to be due to lymphosarcoma, Hodgkin's disease and metastatic carcinoma involving the mesenteric lymph nodes in which the clinical symptoms of steatorrhea were present, but the anatomical pattern demonstrated in the lymph nodes in this condition was not present.

I am glad to hear of the experience of Dr. Robinson in regard to ligation of the thoracic duct. The thoracic duct was dissected out in the case reported by Whipple and the 2nd case of Blumgart's series and no obstruction was demonstrated. We have found no cases in the literature in which obstruction of the thoracic duct has produced the picture we have demonstrated in this case.

EXPERIMENTAL OBSERVATIONS ON THE PATHOGENESIS OF HYPERTENSION.*

Harry Goldblatt, Cleveland, Ohio.

Abstract. A summary of investigations on experimental hypertension due to renal ischemia carried out by the author and collaborators as well as other

* By invitation of the Council.

investigators. (See Harvey Lecture, 1937–1938, page 237, reprinted in *Bull. New York Acad. Med.*, 1938, **14**, 523.)

By constricting the main renal arteries by means of a special clamp devised for the purpose, persistent hypertension was produced in dogs and monkeys which resembles human essential hypertension. The elevation of blood pressure is not immediate, but usually manifests itself in about 24 hours after the production of renal ischemia. Other investigators have now produced hypertension in rabbits and rats by the same method. Some of the dogs have had hypertension for more than 6 years. The type of hypertension which results depends on the degree of constriction of the renal arteries. When one main renal artery is constricted the blood pressure also becomes elevated, but the hypertension does not usually persist for more than a few weeks. In order to make the hypertension persist it is necessary also to constrict the other main renal artery or to remove the other kidney. In animals with hypertension due to constriction of one main renal artery the blood pressure falls to normal in 24 hours, or less, if the ischemic kidney is removed or if the clamp is released. When the renal ischemia is moderate there is no accompanying disturbance of renal excretory function, and the experimental hypertension resembles the benign phase of human essential hypertension. When the constriction of both main renal arteries is marked the excretory function of the kidneys is reduced and degenerative, necrotizing and inflammatory lesions of the arterioles develop in various organs. This resembles the malignant phase of human essential hypertension and also eclampsia.

Moderate constriction of the abdominal aorta, just above the site of origin of both main renal arteries, causes little or no immediate rise of blood pressure, but in about 24 hours hypertension develops without accompanying disturbance of renal excretory function. If the constriction is very great there is disturbance of renal excretory function, as well as hypertension, and pathological changes develop in the arterioles of many organs similar to those following great constriction of both main renal arteries. Constriction of the abdominal aorta just below the origin of both main renal arteries is not followed by elevation of blood pressure, either immediately or later. Thus, in dogs, the hypertension induced by the constriction of the abdominal aorta just above the main renal artery also appears to be of renal origin. Rytand, who worked on rats, came to the same conclusion about the origin of cardiac hypertrophy which developed after constriction of the abdominal aorta above the renal arteries.

The investigations that have dealt with the pathogenesis of experimental hypertension due to renal ischemia have eliminated a nervous reflex mechanism from the kidney as the cause of the hypertension. The present conclusion is that a humoral mechanism of renal origin is responsible for the increased peripheral resistance that determines the elevation of blood pressure. What the nature of the responsible substance is, how it is formed, and exactly how it acts are not yet known. The development of the hypothetical effective chemical substance is presumed to be due to the deficient irrigation of kidney tissue with blood. Whether some substance originally in the blood becomes altered, or accumulates in undue quantity, or whether something is imparted to the blood by the kidney tissue is not yet known. The part played by the organs of internal secretion known to produce pressor substances is not yet elucidated, but there is some indication that the integrity of a portion of adrenal cortex sufficient to sustain life is necessary if hypertension due to

renal ischemia is to persist. The same cannot be stated unequivocally for the hypophysis.

OBSERVATIONS ON THE EFFECTS OF RENAL ISCHEMIA IN PREGNANT DOGS AND RABBITS. C. C. Erickson and L. V. Dill (by invitation), Durham, N. C.

Abstract. Utilization of the Goldblatt clamp technic offered a method of studying the effects of renal ischemia in pregnant dogs. In addition to the expected hypertension and the renal lesions of ischemia, an increased susceptibility of the pregnant animals to renal ischemia was indicated, and lesions of the liver were demonstrated at autopsy.

A comparable syndrome — renal ischemia in pregnancy — has been studied in rabbits. Constriction of the renal artery was produced by a silver wire loop. Control animals included non-pregnant females, pseudopregnant females, ovariectomized rabbits, and pregnant and non-pregnant rabbits with complete arterial constriction. The significance of pregnancy associated with renal ischemia was emphasized by the greater susceptibility of the pregnant rabbits as indicated by survival time. Conspicuous lesions of the liver were demonstrated more frequently in the pregnant rabbits.

The observations suggest a correlation between the physiological and pathological processes induced by renal ischemia in pregnant animals with those in human eclampsia.

Discussion

(Dr. E. T. Bell, Minneapolis, Minn.) I think this is an important contribution to the study of eclampsia, and I think it can be compared to pregnancy in a person with a high degree of renal insufficiency. The most delicate functional test of the kidneys is pregnancy. If a woman has a latent chronic glomerulonephritis and becomes pregnant, the phenomena of eclampsia develop very readily. I think what Dr. Erickson has done is to produce a moderate renal insufficiency, which is entirely comparable with a low grade of chronic glomerulonephritis, and that this brings on the picture of eclampsia. The symptoms of eclampsia are almost identical with those of renal insufficiency.

(Dr. Harry Goldblatt, Cleveland, Ohio.) I purposely omitted any reference to the experiments on this subject which have been carried out by Dr. J. R. Kahn and myself, because I knew that this paper would follow my talk. We have allowed hypertensive animals to become pregnant and have found that the blood pressure has tended to go down, rather than up, during the pregnancy. After parturition the blood pressure has risen again to the previous hypertensive level, or even higher. Although it is true that in a pregnant dog one can produce all the lesions described by the authors, which are similar to the lesions of the "malignant phase" we have already described, yet it is equally true that we have observed the development of identical lesions in uremic and hypertensive non-pregnant females, and even in males, after excessive constriction of the main renal arteries. That the pregnant animal requires less constriction of the main renal arteries in order to produce these lesions, we can neither confirm nor deny on the basis of our experience up to the present time. As a possible explanation for the fall of pressure which we have observed in hypertensive dogs that have become pregnant, we have suggested that perhaps the fetuses with their normal

kidneys may be compensating in some way for the effect of the chemical substance which is responsible for the hypertension, as in the case of unilateral renal ischemia with the other kidney normal. Our own view at present is that there is nothing specific about the pregnancy that is responsible for the development of the eclamptic lesions in animals in which the malignant phase of experimental hypertension is produced by excessive constriction of the main renal arteries. In most other respects our results agree with those reported by Dr. Erickson and Dr. Dill.

(Dr. J. Loesch, Oneonta, N. Y.) About 14 years ago I produced persistent hypertension in dogs. Some of the animals became pregnant during the experiment. As marked nitrogen retention occurred, I fed these animals a low protein diet. Thus a load was taken away from the kidneys with the result that the animals did not die but survived. Otherwise they would have died from uremia. My work agrees with the observation of Dr. Erickson and Dr. Dill. The rest of my experiment showed about the same results as Dr. Goldblatt reported, whose paper I will discuss later.

(Dr. H. Edward MacMahon, Boston, Mass.) It would appear from the pictures Dr. Erickson has just shown us that he and Dr. Dill have successfully produced lesions in the vascular tree of the kidney resembling those seen in fatal chronic nephritic toxemia of pregnancy. The lesions within the liver are also of interest as they resemble those seen in eclampsia and rarely in uncomplicated malignant nephrosclerosis. Gross lesions of the liver in malignant nephrosclerosis are not common. A histological study of the liver in cases of malignant nephrosclerosis does show that this organ is quite commonly affected. Degeneration of isolated liver cells and active regeneration, as shown by the presence of numerous mitotic figures within the liver cells, are more commonly seen than the characteristic vascular lesions. Recently I had the opportunity to study sections from the liver of a middle aged male dying from malignant nephrosclerosis. Under low magnification little or nothing unusual could be seen within the liver cells. A more careful study demonstrated many mitotic figures in liver cells in all sections examined.

(Dr. Harry S. N. Greene, Princeton, N. J.) There is a spontaneous disease in the rabbit which bears a remarkable resemblance to eclampsia. In the rabbit, however, the blood pressure is lowered and the changes in the kidney are entirely degenerative in character. Such differences must be taken into consideration in attempts to induce eclampsia in this animal.

(Dr. Erickson, closing.) The only point I wish to comment on is Dr. Greene's mention of spontaneous eclampsia in rabbits. Certainly many of the lesions of the liver which he has described are quite similar to those we have seen in the pregnant rabbit with renal artery constriction. In this connection it is of interest that a similar spontaneous syndrome has been reported in one or two other animals, guinea pigs and sheep. In dogs, however, there is no similar spontaneous complex. Certainly the puerperal mastitis in dogs, which is sometimes described as an eclamptic complication, is not the same.

VASCULAR HYPERTENSION OCCURRING IN CASES OF COARCTATION OF THE AORTA. Hugo A. Freund, Detroit, Mich.

Abstract not received.

HYPERTENSION AND KIDNEY LESIONS PRODUCED BY X-RAY. F. W. Hartman, Detroit, Mich.

Abstract. As early as 1921 Romberg pointed out that where the maximum systolic blood pressure was constantly above 160 mm. Hg. kidney disease might be the cause. Fahr in 1925 expressed the view that hypertension was a compensatory mechanism and ran parallel with the amount of arteriolar damage in the kidney. In the same year Jaffé stated that there are many cases of hypertension with an isolated sclerosis of the small arteries of the kidney.

In communications on experimental nephritis produced by x-ray from 1925 to 1928, sclerosis of the smaller arterioles of the kidney, hypertension and cardiac hypertrophy were described.

Although an isolated kidney lesion as a cause of hypertension is now widely accepted, there is a definite need for a readily produced experimental renal arteriolar sclerosis in the study and solution of all the problems which this acceptance serves to emphasize.

Using smaller and repeated exposures of x-ray it has been found that the extensive fibrosis and replacement of all kidney structure as shown in previous work can be avoided. However, sclerosis of the intermediate and smaller arterioles, resulting in hypertension, cardiac hypertrophy, and finally kidney insufficiency, is produced.

HYPERTENSION FOLLOWING EXPERIMENTAL PERINEPHRITIS INDUCED BY CELLOPHANE. A PRELIMINARY REPORT. Irvine H. Page (by invitation), Indianapolis, Ind., and Irving Graef, New York City.

Abstract. It has been found that sterile cellophane, when gently applied, wrapped around the kidneys of dogs and secured either with paper clips or loose ligatures, produces an intense inflammatory reaction followed by a continuous fibroblastic and collagenous deposit. The inflammatory response produces a constrictive capsule 3 to 5 mm. thick around the kidneys. After several months the hilar structures, especially the renal vein, pelvis and ureters, may also be compressed.

From 2 to 3 weeks after the application the arterial pressure (measured by direct intra-arterial puncture) begins to rise and may reach a level of 240 mm. Hg. mean pressure after a month or two. In some animals the pressure reaches a peak and tends to fall to lower levels, while in others the pressure remained at high levels for 7 months (as long as the animals were being observed). Application of cellophane to one kidney caused hypertension, but not as marked as when both kidneys were treated.

At postmortem examination the kidneys, in short experiments, were found to be surrounded by a sanguinopurulent exudate in the form of a membrane between the true capsule and the cellophane. A similar exudate is present on the outer aspect of the cellophane. After 2 weeks the exudate around the cellophane continues to be sanguinopurulent and a dense fibroblastic and collagenous deposit appears on the true capsule. This increases to 3 to 5 mm. in thickness and extends around the entire kidney. At the end of 2 to 3 months the constrictive effect is marked and the kidney may be rotated in any plane and the hilar structures distorted and compressed.

The omentum becomes attached to the outer aspect of the cellophane and the exudate on the kidney. Dense adhesions which are richly vascularized and

the seat of marked histiocytic proliferation bind the kidney and the omentum together. Histological examination of the kidney reveals negligible changes in the first 2 months. Later, in the period of observation thus far used, marked compression with focal corticotubular atrophy and cortical scarring of the ischemic type become apparent. This is sometimes visible macroscopically, but is more marked on microscopic study. The glomeruli are remarkably well preserved in most instances. The collagenous hull which forms external to the normal capsule is readily separated from it and the true capsule shows little or no change. It in turn may be readily stripped from the underlying parenchyma.

The fate of cellophane is as yet undetermined. After long sojourn it may be found crumpled or broken into large pieces caught between dense fibrous deposits. When removed it appears to be unaltered and the inflammatory response persists around it. There is remarkable lymphoid hyperplasia in the nodes of the omentum; sometimes they exhibit acute lymphadenitis.

Hypertension produced by this method occurs whether the normal capsule is stripped or not before the application of cellophane. Denervation of the kidneys also does not interfere with its development. Removal of the "offending" kidney in animals in which hypertension has occurred after applying cellophane to one kidney causes the hypertension to disappear if it has not persisted for a long time.

Since hypertension may appear as early as 10 days after the application of cellophane to a kidney, it seems likely that the mechanism of its production is independent of gross compression of the renal artery.

EXPERIMENTAL ACUTE HYPERTENSION FROM OBSTRUCTION OF THE AORTA. Robert Brotchner (by invitation) and E. T. Bell, Minneapolis, Minn.

Abstract. Goldblatt found that constriction of the aorta above the kidneys produces chronic hypertension, while constriction below the kidneys does not. The hypertension resulting from constriction of the aorta above the kidneys is attributed to renal ischemia.

This idea of renal ischemia as a cause of hypertension has been extended by some writers to include the hypertension in the upper extremities which results from coarctation of the aorta.

Using dogs under nembutal anesthesia the blood pressure was recorded on a kymograph with a cannula in the carotid artery. When the aorta was clamped above the origin of the celiac artery the blood pressure rose immediately, the average rise being 56 mm. Hg. On release of the clamp the blood pressure fell immediately to its previous level. When the aorta was clamped between the renal arteries the average rise of blood pressure was 13.5 mm. Hg., and when clamped below the kidneys, 8.8 mm. Hg. Clamping of both renal arteries did not affect the blood pressure.

It is concluded that the sudden rise of blood pressure following constriction of the aorta is due to a mechanical factor and not to renal ischemia. It is noteworthy that the degree of hypertension is directly related to the amount of blood obstructed. According to the work of Levy and Blalock, 60 per cent of the blood is obstructed by a clamp applied above the celiac artery and only 13 per cent by similar obstruction below the kidneys.

It is inferred that congenital stenosis of the aorta at the isthmus (coarctation) produces hypertension by mechanical obstruction of the circulation and not by renal ischemia.

VASCULAR MEASUREMENTS IN HYPERTENSION. H. E. MacMahon, Boston, Mass.

Abstract. The problem was divided into six parts: (1) to make an anthropometric study of the arterial tree of the kidney in the non-hypertensive state; (2) to make a similar study in conditions showing acute transient hypertension; (3) to make a similar study in several of the chronic hypertensive states; (4) to compare the vascular measurements in the hypertensive state with those in the non-hypertensive state; (5) to compare the vascular measurements in the arterial tree of the kidney in benign and malignant nephrosclerosis; and (6) to evaluate the significance of these anthropometric findings on the maintenance and course of prolonged and lasting hypertension.

This work was based on an anthropometric study of the arterial tree of the kidneys from 100 individuals ranging from 20 to 65 years of age. These were divided into three groups: (1) those with average blood pressure readings; (2) those with transient and acute hypertension including acute glomerulonephritis, acute hyperthyroidism and eclampsia; and (3) those with prolonged and lasting hypertension, including subacute glomerulonephritis, subchronic glomerulonephritis, chronic glomerulonephritis, benign nephrosclerosis and malignant nephrosclerosis.

In the preparation and selection of material, two or more blocks were cut from the kidneys in each case. The handling of the tissues was kept as constant as possible, and to facilitate measuring, differential stains on serial sections were frequently employed.

Measurements were made with a small micrometer eyepiece set into the ocular of the microscope. For simplicity, and since the study was primarily of a comparative nature, all measurements were merely read as "measuring units," rather than in microns. Approximately 40 transections of vessels were measured in each case. Five measurements were made from each transection, comprising: (*a*) the total width, (*b*) the diameter of the lumen, (*c*) the thickness of the wall, (*d*) the media, and (*e*) the intima. The total number of measurements was well over 20,000.

From these measurements a mean-average arterial tree for each case was graphically constructed, and on the basis of these measurements and graphs a comparative study of the several groups was made possible.

A brief summary of the outcome of this work is as follows:

Anthropometric studies on the arterial tree of the non-hypertensive and acute transient hypertensive groups were alike and may be represented by identical curves.

In the non-hypertensive group the artery shows a slow, gradual progressive diminution in size in its course through the kidney, maintaining an "angle of convergence" of approximately 15°. The lumen, the wall, the intima and media likewise diminish gradually and slowly. The diameter of the lumen is always greater than the thickness of the wall. The relative thickness of both wall and media is slightly greater in the arteriole than in other portions of the arterial tree. The intima reaches its relative and absolute peak in the larger vessels.

In the chronic hypertensive group the vessels are large throughout and show a rapid decline with an "angle of convergence" of 35°. The lumen of the arteries is large, uneven and precipitous. In the arterioles the lumen is narrow and stenotic and its diameter is very much less than the thickness of

the wall. The wall throughout is thick and heavy. The media in the larger vessels shows eccentric hypertrophy, whereas in the arterioles the media is insignificant, stretched and nearly lost. The intima is thick throughout and rather uneven. It attains its relative and near absolute peak in the arterioles and smaller arteries respectively.

A comparison of the vascular measurements in the chronic hypertensive and non-hypertensive groups brings out several striking differences.

In chronic hypertension the arterial tree is larger and pursues a comparatively precipitous course, with a larger lumen in the artery and a smaller stenotic lumen in the arteriole. The wall throughout is thicker and reaches a greatly exaggerated relative peak in the arterioles and smaller arteries. The media of the larger vessels is much greater, the media of the arterioles is much less. The intima is thicker, less uniform throughout, and reaches its relative greatness in the arterioles and smaller arteries, in striking contrast to the relationships in the non-hypertensive group.

A comparison of the vascular measurements in benign nephrosclerosis and malignant nephrosclerosis shows many striking similarities. The vessels are very large and precipitous. The lumens follow a rapidly converging course, being very large in the large vessels and very narrow in the arterioles. The walls are large and thick. The intima reaches its relative peak in the arterioles. The media of both groups is identical. The differences in the two groups (benign nephrosclerosis and malignant nephrosclerosis) were slight. The intima of the arteriole and smaller artery in malignant nephrosclerosis is 30 per cent thicker than that of benign nephrosclerosis, and the lumens of the same size vessels are somewhat smaller.

The significance of these anthropometric findings in the mechanism of hypertension and especially in the maintenance and course of prolonged and chronic hypertension is as follows:

Acute and transient hypertension may be found in cases in which the vascular tree is normal. That is to say, a disturbed anthropometric relationship in the arterial tree is not a prerequisite to hypertension.

With prolonged hypertension there is first an increase in size of the arterial tree which varies with the degree and duration of hypertension. This increase in size is the result of a progressive dilatation of the lumen accompanied by an eccentric hypertrophy of both intima and media.

In prolonged hypertension the "angle of convergence" is progressively increased, permitting a greater force to strike the smaller branches of the arterial tree.

The intima of the arteriole then thickens, leading to a progressive stenosis of its lumen. This increases the peripheral resistance and aggravates the conditions already existing. An increasingly vicious circle is gradually set in motion to maintain and to increase the existing hypertension.

CHANGES IN THE LARGER ARTERIES ASSOCIATED WITH EXPERIMENTAL HYPERTENSION AND AZOTEMIA. L. L. Waters (by invitation) and M. C. Winternitz, New Haven, Conn.

Abstract. The lesions of arterioles associated with hypertension and azotemia as produced and described by Goldblatt have their counterpart in the larger arteries and veins. Here the medial elastic fibers are spread apart and blood is found extravasated from vasa made conspicuous by necrosis of their walls

and the immediately surrounding tissues. These experimentally produced lesions are of particular interest in view of the occurrence of hemorrhage, necrosis and fibrosis of the media of the human aorta associated with renal hypertension and nitrogen retention.

Discussion

(Dr. Theodore J. Curphey, Westbury, N. Y.) I should like to ask whether those scars in the media showed perivascular round cell infiltration.

(Dr. Howard T. Karsner, Cleveland, Ohio.) I am much gratified to hear what Dr. Winternitz says about these lesions in the media because in studying a large number of aortas I have been deeply impressed by the fact that such things do occur, and fairly frequently. Thus his experience coincides with mine. I may, I hope, be permitted to make reference to Dr. Curphey's question. Not infrequently these lesions are accompanied by what is loosely called "round cell infiltration," but we should be more specific in identification of the "round cells." Cellular infiltrations in the neighborhood of syphilitic lesions are somewhat different from those found in these lesions. In syphilis the cells are principally lymphocytes, and in these other lesions, although lymphocytes are present, large mononuclear cells predominate. Plasma cells may be found in both.

(Dr. Winternitz.) There is one thing: we see very little active syphilis, and after it has subsided there is a scar, and with due deference to my old teacher, Dr. MacCallum, I agree one cannot tell whether it is due to a spirochete or a mule kick, but when the lesions are active I think it is definitely true, as Dr. Karsner says, that the cytology in the vicinity of the lesion is very helpful.

Discussion of Papers on Hypertension

(Dr. Howard T. Karsner, Cleveland, Ohio.) At the 1938 meeting of the Association of American Physicians I reported, on the basis of measurement of the thickness of the aortic media in nearly 500 cases, that the media of hypertensives is definitely thicker than that of non-hypertensives in comparable age groups. The increased thickness is associated with a separation of the elastic lamellae and a greater degree of intricacy of pattern than is found in the controls. Since advanced age is accompanied by much the same changes, it was suggested that perhaps hypertension produces a precocious senility in the aorta. Haythorn and others have determined the gradual increase in amounts of calcium in the aorta as age advances. Sections of the same aortas previously reported were treated by Kóssa's silver nitrate method and examined for calcium. If the increased thickness of the aortic media were due to precocious senility, it is to be expected that the aortas of hypertensives would show a greater degree of calcification than the controls. Roughly estimating the calcification as absent, moderate or marked, admittedly only a rough quantitative determination, it occurs in about the same degree throughout all the decades in both hypertensives and non-hypertensives. Thus it can be stated that the increased thickness of the aortic media in hypertension is not a manifestation of precocious senility. Evidently other factors determine the increased thickness of the aortic media in the human subject of hypertension.

(Dr. J. Loesch, Oneonta, N. Y.) I was very much interested in Dr. Goldblatt's paper because about 14 years ago I did similar experiments myself.

They were reported at the meeting of the Federation of Biology, Section of Experimental Pathology, in Rochester, N. Y., in 1926, and published in the February 18th and 25th, 1933, issues of the *Zentralblatt für innere Medizin.*

The approach of my experiment, however, was slightly different. In 1925 I did some work on extirpation and exclusion of the spleen, and during this experiment I found it of advantage to transplant the spleen under the skin. This gave me a clue to apply the same method to the kidneys. I transplanted both kidneys under the skin, or one kidney, and removed the other later if a "one kidney dog" was desired. To assure quick healing all pericapsular fat and that around the kidney pedicle was removed, probably also all nerves. Later I made an incision on both sides of the pedicle and clamped the renal artery, probably with the latter also frequently the veins, for various lengths of time every 3rd day, beginning at 5 minutes and gradually increasing to 30 minutes. By doing this a fibrotic ring was formed around the vessels, resulting in ischemia. The latter was, however, marked during clamping, which was intended to imitate spasms in human beings. The blood pressure in these experiments rose gradually within a month to 200 mm. Hg. or higher.

I followed these experiments for about a year and the blood pressure remained elevated even when for several months no clamping was done. The diastolic pressure rose, although somewhat later, to about 90 mm. Hg. In the one kidney animal the blood pressure reached about 180 mm. Hg. or slightly above. I thought these experiments gave an explanation for the etiology of hypertension, at least in some cases, although Kylin had denied any relation of the kidneys to hypertension. On the one hand, Romberg was a proponent of the renal theory. Volhard explained it on spasms, and Fahr on ischemia; thus I was able to prove by these experiments that the renal and ischemic theory was right. In my experiments the increase in blood pressure was caused by a renal and extrarenal, but renally determined, factor. If in one kidney dogs clamping was done 30 minutes every day over various lengths of time, they died from uremia. If, however, I clamped the two kidney dogs, which were fed the same diet, they lived much longer or did not die at all. The histological changes were about the same as Dr. Goldblatt reported; thus I found marked cellular proliferations of the glomerular capsule and loops, and further, fibrosis and hyaline degeneration of the glomeruli. The smaller arterioles, especially the afferent vessels, revealed marked thickening of the wall with nearly complete obliteration of the lumen. The tubules showed distention, flattening and desquamation. The heart was markedly hypertrophic and the brain edematous, but the rest of the organs showed hardly any changes. My conclusion at that time was that I was able to produce experimentally persistent hypertension.

(Dr. Paul Klemperer, New York City.) May I ask to what Dr. Goldblatt attributes the return of the blood pressure to normal after unilateral constriction of the renal artery. Is it due to the development of collateral circulation, so that if one would be able to avoid the formation of collateral circulation the blood pressure would be retained, or do you attribute it to the excretion of a hypothetical pressor substance by the normal kidney?

(Dr. E. T. Bell, Minneapolis, Minn.) I want to congratulate Dr. Goldblatt on his brilliant work. He has presented it to us very modestly. He does not claim that he has solved the problem of primary hypertension. I hope we won't rest on our oars and conclude that this whole question has been settled. The argument has been going on for 25 years as to which is first — the vascular

disease or the increase in blood pressure. There are certain arguments in favor of the idea that hypertension is primarily due to vascular spasm. One of these is that brought out by Dr. MacMahon, that there are clinical cases of primary hypertension similar in all respects to ordinary cases in which there are no appreciable changes in the vascular bed of the kidneys. There may of course be different kinds of hypertension. There are other arguments. Cases of long standing chronic glomerulonephritis often show an arteriolosclerosis of high degree. Unless we think the two diseases are associated, we must conclude that the prolonged hypertension has caused the arteriolosclerosis. There is the further consideration that the disease in the kidneys in hypertension begins in the large arteries and progresses toward the smaller vessels. We never see arteriolar disease without disease of the larger arteries preceding it. There is the further consideration that in the early stages of primary hypertension the patient during sleep has a fall in blood pressure, indicating that a vasomotor phenomenon is concerned in the disease.

(Dr. Goldblatt, closing.) Mr. Chairman, it is impossible to give you an adequate discussion of the interesting papers presented this morning in the 5 minutes that have been assigned for this purpose, but I shall do my best.

Dr. Fox whispered to me, "What do you think is the nature of the humoral mechanism which you consider responsible for the hypertension?" I do not know the answer, of course, but I can say that I am now a believer in the humoral mechanism and think that something happens to the blood that is going through a deficiently irrigated kidney which results in the presence in it of some chemical substance or property that can effect increased peripheral vascular resistance. Whether it means that some part of the kidney actually contributes a pressor substance to the blood, or that a pressor substance which is ordinarily eliminated accumulates in the blood, or that a substance in the blood that is not ordinarily pressor is changed to a pressor substance by flowing through the deficiently irrigated kidney, or that it is due to the neutralization, elimination, or destruction of a natural depressor substance, I do not know; but these are at least some of the possibilities. I can assure Dr. Fox that all of these possibilities are being investigated with great care by ourselves and many other workers.

I have already discussed the paper by Dr. Erickson and Dr. Dill, but I would like to add that we have found that the development of pregnancy in dogs with renal ischemia, in which the elevated blood pressure had returned to a lower level, did not cause the re-elevation of the blood pressure. In order to re-elevate the pressure in such dogs, it was found necessary to increase the renal ischemia. The dog is a quadruped with abdominal contents hanging down, so that in the usual positions assumed by dogs a mechanical compression of the aorta, or increased constriction of the arteries and veins, is not likely to result from the pressure of a pregnant uterus. In a human female, in the last stages of pregnancy, when the uterus is greatly enlarged, it is at least possible that acute renal ischemia resulting from pressure on the aorta and main renal vessels may be the cause of the hypertension and renal excretory insufficiency characteristic of this condition. The fact that eclampsia usually develops very late in pregnancy is certainly suggestive. We have even dared to suggest that pregnant women in the state of eclampsia might be placed on a contrivance which would permit the uterus to hang down and thus relieve the acute renal ischemia that might exist. Dr. Weiser of Detroit has already tried this procedure in a few cases with apparently good results.

In regard to Dr. Freund's contribution, in which he arrived at the conclusion that the hypertension which accompanies coarctation of the aorta, even in the thorax, may be due to renal ischemia, I can only say that all of our attempts to produce persistent hypertension in dogs by constriction of the aorta within the thorax have been without much success due to various complications. We hope now by a different method to be able to study this problem. However, I do believe that the hypertension which occurs in the upper part of the body of dogs with the aorta constricted just above the main renal arteries in the abdomen is of renal origin. Freund mentioned that in young individuals with coarctation of the aorta in the thorax they found little or no cardiac hypertrophy. This interests me, because the young are not apt to have significant coronary arteriosclerosis with resultant myocardial ischemia which probably plays a part in the greater cardiac hypertrophy observed in older people with persistent hypertension from any cause. We have found that in dogs the degree of cardiac hypertrophy as a result of persistent hypertension is also not great, and have felt that a possible explanation may be the absence of significant coronary arteriosclerosis in the dog.

Dr. Hartman's contribution is valuable. That intrarenal arteriolar and glomerular disease is produced by the irradiation is important. The only drawback of the method is that it is difficult to control the degree and extent of pathological changes in the kidney, and that, like our animals in the malignant phase, Dr. Hartman's dogs usually die of renal excretory insufficiency. This does not offer an opportunity for the study of the benign phase of experimental hypertension which, in my opinion, is the more important phase.

The contribution of Dr. Page and Dr. Graef is also of considerable interest. The hypertension which follows the application of an envelope of cellophane around the kidney is due in all probability to compression of the kidney substance by the thick perirenal scar which forms, as well as to compression of the renal vessels at the hilus, both of which may produce renal ischemia. There may even be interference with venous outflow from the kidney, which Dr. Bell and Dr. Pedersen showed years ago may be a cause of at least temporary hypertension. In order to reduce the accessory circulation to the kidney we have used dried sheep's cecum to form a membrane around the decapsulated kidney. This did not induce the formation of such a thick perirenal scar as is produced by the envelope of cellophane, and reduced the accessory circulation to the kidney, which was our only purpose.

In commenting on the contribution of Dr. Brotchner and Dr. Bell, I can only say that while it is true that I may not have committed myself very definitely in my talk in favor of the view that the hypertension which develops after constriction of the aorta just above the main renal arteries in the abdomen is due to renal ischemia, yet I believe this to be the case. My own interest in ischemia goes back to 1923, when I was a pupil of Professor Starling and worked under the direction of Dr. Anrep in the Department of Physiology at University College, London, England. My first studies dealt with ischemia of the legs of dogs and the reactive hyperemia which follows the return of the normal circulation to the ischemic limb. In studying this problem it became necessary to determine the effect of clamping the aorta at various levels right up to its origin from the heart. I found what had been known to physiologists for a long time, namely, that if one clamps the aorta at various levels, the immediate effect is a rise of the blood pressure above the site of the clamp and an abrupt fall of the blood pressure below it. The more

cephalad the site of the constriction of the aorta, the greater the immediate rise of blood pressure above the site of the clamp. This is in keeping with Dr. Bell's results. Had Dr. Brotchner and Dr. Bell clamped the aorta within the thorax, the immediate rise of blood pressure above the site of the constriction would have been even greater. Their longest period of observation lasted 40 minutes. In order to conclude that the hypertension above the site of the clamp as a result of the constriction of the aorta is entirely of mechanical origin, they should have studied the effect of the blood pressure for a much longer period. I believe they would have found that the initial immediate hypertensive effect would have disappeared in a short while, and that only 24 or more hours later would the blood pressure have again risen and remained elevated at least for a few weeks. I do believe that the immediate effect described by Dr. Brotchner and Dr. Bell is entirely of mechanical origin and due to the sudden interference with the onflow of blood through the aorta. The later rise of blood pressure which occurs after 24 or more hours, as in the case of the hypertension which follows clamping of the main renal arteries, is in my opinion due to renal ischemia. As a matter of fact, by very great constriction of the aorta, one may actually produce renal excretory insufficiency, as well as hypertension, and all the arteriolar lesions of the malignant phase that can be produced by constricting the main renal arteries.

At this stage I may state that those who do not accept the renal origin of the benign phase of essential hypertension are usually willing to accept it for the malignant phase. I know this to be true in the case of Professor Volhard. In my opinion this last concession is not necessary, for all that need be admitted is the renal origin of the renal excretory insufficiency. Yet I do believe that both phases can be of renal origin.

I can make no special comment on the study of Dr. MacMahon, not having made a similar study. His conclusion is different from that of Moritz and Oldt, on the basis of their study, with which I agree. We therefore obviously have diametrically opposite views, either of which may yet be proved true by experiment but cannot be settled by discussion.

The paper by Dr. Waters and Dr. Winternitz is an illustration of a very careful study in morbid morphology. Their findings are in keeping with the little that we can contribute to this subject on the basis of an entirely inadequate study up to the present time. Dr. Winternitz, with his great interest in the pathogenesis of arteriosclerosis, is the right man to make a study of the changes which occur in the large vessels of animals in the benign and malignant phases of hypertension due to renal ischemia. I look forward to some valuable contributions from his department on the causative relations between the hypertension, the renal excretory insufficiency, and the changes in the aorta and the large blood vessels.

In reply to Dr. Klemperer's question as to why the blood pressure returns eventually to normal when only one main renal artery is constricted, I can say that there are at least two possible answers. One possibility is that the natural accessory circulation to the ischemic kidney becomes sufficiently prominent to compensate for the ischemia. It has been known for many years that the dog has an abundant potential accessory renal circulation through the capsule and from other sources which may become prominent and effective under these conditions. The other possible explanation is that the normal kidney may compensate in some way by eliminating or neutralizing the effect of the hypothetical effective substance that is responsible for the increased

peripheral resistance. Both factors may account for this phenomenon.

In the time that was allotted to me for my own talk, it was impossible for me to cover the entire ground and to refer to all the experiments that have been done to elucidate the pathogenesis of essential hypertension. However, I would like to take this opportunity to mention the work of Homer Smith and Goldring which is exceedingly interesting to me. Their study, by indirect methods, of renal blood flow in cases of human essential hypertension, indicates that there is always some reduction of blood flow through the kidney. This constitutes valuable support to the view that renal ischemia may, after all, be the initial cause of both the benign and the malignant phases of essential hypertension.

EXPERIMENTAL INFECTIOUS PHLEBITIS AND ARTERITIS. P. M. LeCompte (by invitation) and M. C. Winternitz, New Haven, Conn.

Abstract. Various organisms, when injected into the wall of the femoral vein in the goat, give rise to a reaction the extent and character of which are determined largely by the virulence of the organism and the duration of the experiment.

The lesions in the wall of the vein vary from acute phlebitis, with or without thrombosis, to fibrous intimal plaques. Particular emphasis should be placed on lesions of the neighboring artery; these consist of early intimal proliferation, exudation with fibrin precipitation in the vicinity of the internal elastic lamella, and later of fibrous intimal thickening.

A possible vascular pathway for such transfer of infection from vein to artery has been demonstrated by injection methods.

BLOOD CHOLINESTERASE IN RHEUMATIC FEVER. Mark P. Schultz and (by invitation) Edythe J. Rose, Washington, D. C.

Abstract. Whole blood and blood serum were obtained at approximately 10 day intervals from 24 patients with rheumatic fever and from 21 suffering from various other febrile diseases during the course of their illness, and these specimens were examined for cholinesterase by the method of Ammon and Voss. The usual range of values was established by examining 31 apparently healthy individuals. During the course of febrile diseases other than rheumatic fever the cholinesterase fell far below this level and invariably failed to return to it until the patients had completely recovered, as indicated, among other considerations, by the absence of fever and the return of the erythrocyte sedimentation rate to within normal limits. In 20 patients with rheumatic fever, on the other hand, the cholinesterase in some instances remained at the usual level or above, while in others it fell during the acute exudative stages of the disease, but remained at usual levels or above during frequently protracted periods of subacute activity associated with progressive carditis. In each of the 6 patients with chorea examined, the values without exception were also unusually high. In 4 only of the rheumatic fever patients observed the cholinesterase dropped and, as in the other febrile diseases studied, failed to attain usual levels until recovery was established. These patients were all young adults who suffered severe arthritis, but none of them developed more than discernible signs of carditis and in none did other than minimal cardiac damage result. Of the 20 rheumatic fever patients

in whom unusually high cholinesterase values were observed, on the other hand, 10 died and severe carditis with resultant extensive cardiac damage was the rule. In rheumatic fever patients a correlation was observed between variations in the P–R interval of the electrocardiogram and in the cholinesterase level. Increases in the former were associated with diminishing values for the latter, and vice versa.

MOTILITY AND CHEMOTAXIS OF LEUKOCYTES IN HEALTH AND DISEASE. Morton McCutcheon and (by invitation) O. Tod Mallery, Jr., Philadelphia, Pa.

Abstract. The outcome of an infection depends in part on the rapidity with which polymorphonuclear leukocytes are mobilized in infected tissues, and this mobilization is possible only as long as leukocytes react to the presence of bacteria by moving toward them. In order to find out whether this reaction is impaired in disease, experiments were made *in vitro* on leukocytes from a series of acutely ill persons suffering from such diseases as pneumonia, typhoid fever and congestive heart failure. For comparison, experiments were made also on leukocytes of the observer and on leukocytes of a series of patients not acutely ill.

A small clump of bacteria — pneumococcus, *Staphylococcus aureus*, or typhoid bacilli (differences in attraction could not be demonstrated) — was placed on a glass slide and a drop of blood, obtained by puncturing the finger, was superimposed and allowed to spread between the slide and the coverslip. The preparation was observed with the microscope at 37° C. A portion of the clump of bacteria was placed in the microscopic field and the path of each leukocyte was recorded on paper with the help of a drawing ocular.

Records made in this way were analyzed to find, first, how rapidly the cells moved, and second, how direct a path they followed toward the bacteria (the directional response of cells is known as chemotaxis). Leukocytes from acutely ill persons were found on the average to move 30 per cent less rapidly than those of the observer and those of patients not acutely ill. Also, cells of acutely ill individuals did not move quite as directly toward the bacteria as did those of the observer. It is concluded from these experiments that in the ill patients both rate of locomotion and chemotaxis were reduced — motility greatly, chemotaxis only slightly. The results indicate that leukocytes were damaged in the acutely ill patients, and this alteration might be expected to hinder the mobilization of leukocytes in infection.

Discussion

(Dr. Theodore R. Waugh, Montreal, Canada.) I should like to ask Dr. McCutcheon if he considered the possibility that the collection of the red cells in the large aggregations which occur in acute conditions might possibly have had some effect on the path of the leukocytes. It is well recognized that in acute conditions there is a rapid sedimentation velocity of the blood and this is attributed to agglutination of the red cells in large aggregations.

(Dr. Theodore J. Curphey, Westbury, N. Y.) Have you tried the leukocytes of the patient in the plasma of the observer to see whether there might be something in the humoral side of the blood that might influence the changes in the leukocytes?

(Dr. Paul R. Cannon, Chicago, Ill.) Is it possible to make any similar measurements of immature leukocytes? I assume that the leukocytes were ma-

ture, but I wonder if immature leukocytes would be attracted at a slower rate, and whether or not you have made any observations of that sort.

(Dr. Howard T. Karsner, Cleveland, Ohio.) Will Dr. McCutcheon take the opportunity to harmonize these observations with the phagocytic activity of the leukocytes?

(Dr. McCutcheon, closing.) The first question was whether the path of the red blood cells might be obstructed by agglutinated red blood cells in ill patients. I can only say that agglutination was not observed — probably it was not especially looked for. It seems to me a possibility that the rate of locomotion would be reduced. I do not think it would be greatly reduced.

The second question concerned the leukocytes of the patient in the plasma of the observer. Unfortunately I have no data on that.

In regard to Dr. Cannon's question on the study of immature leukocytes, I think this could very readily be done in rabbits by injecting saline solution into the peritoneal cavity, as Ponder has shown that if this is done repeatedly at short intervals the blood contains many immature leukocytes. Then it would be possible to compare their chemotactic response with that of mature cells.

In regard to Dr. Karsner's question, we have made no comparison of these studies with phagocytosis. I think it would be very interesting to do so.

THE PATHOLOGY OF HUMAN BRUCELLIASIS.* P. B. Parsons, Mary A. Poston, and Bowman Wise (by invitation), Durham, N. C.

Abstract. In November, 1937, a report of pathological investigation of 4 cases of human brucelliasis was made in a preliminary form before the Section on Pathology of the Southern Medical Association. In the 1st case complete clinical investigations were followed by complete autopsy. The other 3 cases were studied clinically and by means of excised enlarged lymph nodes. The 1st case came under observation presenting a clinical picture with laboratory findings indicative of infection with *Brucella melitensis*. This diagnosis was confirmed by culture of the organism from excised lymph nodes. The histological picture of the lesion of the lymph nodes was that of non-specific necrosis. At autopsy the lesions were those of Hodgkin's disease grossly and histologically, but *Brucella melitensis* was cultured from numerous lesions in pure culture. The other 3 cases were studied clinically and by means of excised enlarged lymph nodes without culture. In these cases the clinical diagnosis on histological study was Hodgkin's disease. In view of the association between the histological and gross lesions of Hodgkin's disease and the organism *Brucella melitensis* in the case studied at autopsy, the 3 cases just mentioned were restudied clinically and other lymph nodes were removed. These nodes obtained at a second operation were studied histologically and bacteriologically. The histological picture was again that of Hodgkin's disease, but from the nodes a pure culture of *Brucella melitensis* was obtained.

This experience suggested naturally a possible relation between chronic brucelliasis and Hodgkin's disease. The work described in this report deals with further studies designed to test the working hypothesis that these two diseases may be of one etiology. Seven cases of clinical Hodgkin's disease have now been studied by biopsy and by culture of excised lymph nodes. Clinical diagnosis of Hodgkin's disease has been histologically confirmed in all 7 cases and from all *Brucella melitensis* has been grown in pure culture.

* This work has been supported in part by the James A. Greene Research Fund.

A series of 50 control cases has been studied by the same technic, but in none of these has the organism of brucelliasis been obtained. One of the original 3 biopsied cases had a third biopsy after a period of about 1 year. The organism was not obtained from the culture of the lymph node, but the histological picture was not changed essentially from that of the originally excised node.

The work here reported is a part of a series of studies of human brucelliasis now in progress. The present state of its development warrants the drawing of but one conclusion, that is, that chronic brucelliasis of the glandular type prevalent in the area in which this work is being done cannot be differentiated from Hodgkin's disease by either histological or clinical methods. Since the histological method has always been regarded as the criterion of diagnosis of Hodgkin's disease, this fact would seem to be significant with regard to the basic nature of these two disease entities.

Discussion

(Dr. Herbert Fox, Philadelphia, Pa.) In view of the fact that millions of lymph glands have been cultured and similar studies made, I wonder what the method for isolation of *Brucella* has been to get 12 per cent positive in these 115 cases. I know I have tried, and others have tried, and millions of lymph nodes of the Hodgkin's type have been cultured. I am afraid I cannot go along with the thought that every large cell which has been called a Sternberg type is necessarily finally diagnostic of that condition we know as Hodgkin's disease, because Hodgkin's disease is not only a group of these cells, but it is a great deal more. I also wonder whether it is fair to take that picture of Sternberg's, which he himself told me might have been tuberculous disease, and compare it with the original ones which Hodgkin himself left.

(Dr William H. Feldman, Rochester, Minn.) I am very much interested in this report and would like to know what were the types of *Brucella* found. Dr. Parsons called them *melitensis*, and I should like to know the criteria for differentiating the type of organism.

I would also like to ask if there was any evidence of spondylitis in patients from which the material was obtained.

(Dr. Charles T. Olcott, New York City.) Were there eosinophils in these lesions of Hodgkin's disease, and were there any fibroblasts?

(Dr. Howard T. Karsner, Cleveland, Ohio.) Without necessarily accepting the validity of Gordon's test, I think it would be of interest to know what results were obtained in this series of cases, if the Gordon test was applied.

(Dr. Fritz Levy, Elkins, West Va.) I would also like to know if eosinophilic cells were found, and what kind of mitoses was found in those large cells. In 1920 I reported in a preliminary note about cell division that characteristically abnormal mitoses were found in the cells which usually are called Sternberg's or Dorothy Reed's cells. They belong to a group of cells which have plurivalent nuclei after abnormal cell division. When at the end of a normal bipolar mitosis the nucleus is divided, but the cytoplasm is not, we get binucleated cells; sometimes the two nuclei fuse and we get large cells with large nuclei of the same type. I agree with the first discussion concerning the point that we are not allowed to make the diagnosis only on these large cells with one large or more normal sized nuclei, because it is only a certain deficiency in cell division. While we find this type of cell often in Hodg-

kin's disease, we find the same type of plurivalent cells in many other conditions when cell division is disturbed. At least we have to make sure that we are dealing with plurivalent cells of endothelial origin.

(Dr. Wiley D. Forbus, Durham, N. C.) I have been much interested in this finding, and there are certain things I should like to say, especially since I have carefully studied all of the material. Dr. Parsons has emphasized the Dorothy Reed type of cell, I think, unduly. He has not said enough of the other features of these tissues which are, in my opinion, identical with those of Hodgkin's disease. Being at first naturally quite sceptical, in order to assure myself of the genuineness of this histological similarity, I took all of the cases of Hodgkin's disease which we have seen at autopsy in the past 10 years and went over them very carefully, grossly and histologically. From a study of all of the records I was unable to make a differentiation between the pathological anatomical picture which Dr. Parsons has shown you, and that presented by the 8 or 9 cases of Hodgkin's disease, that is, those which we have called Hodgkin's disease. There is the possibility that we do not know what Hodgkin's disease is. We are fully aware of the fact that there are a great many different stimuli which are capable of provoking this particular reaction in lymph nodes. That *Brucella* may be one of those agents I think there is no question. Whether or not what Dr. Parsons has presented to you is Hodgkin's disease is something which, frankly, remains to be proved. We are not resting on our haunches in the matter since the importance of determining the true significance of the striking pathological similarity between brucelliasis as shown to you by Dr. Parsons, and Hodgkin's disease as we have all seen it, is obvious.

(Dr. Ralph D. Lillie, Washington, D. C.) I should like to ask a question as to whether there was the characteristic reticulum deposit among these pale cells we see in the usual type of Hodgkin's disease.

(Dr. Parsons, closing.) The first question was on the culture methods. Miss Poston has done most of the culture work and has worked with it for many years. This is the method which she uses: beef infusion blood agar adjusted to pH 7.4. We also use liver infusion agar with blood added at the time the culture is planted adjusted to a pH of 6.8. No culture is considered negative until it has been given a trial for at least 2 weeks, both aerobically and anaerobically.

The second question is one which several people asked. I am sorry I did not mention that in addition to the Dorothy Reed or Sternberg cells there were great numbers of eosinophils present and considerable fibrosis throughout the nodes. There was none of the original or usual architecture one would expect to find in lymph nodes. There were a great many large reticulum cells which we see in Hodgkin's disease. These nodes have been considered as Hodgkin's disease, not only by members of our department but by others outside of Duke University.

In regard to the question about the identification of the organism: The first culture was identified as *suis*, and three of the others as the bovine variety by both the agglutination and by the agglutinin-absorption method. They were sent to the National Laboratories for identification. The rest have been identified by us by simple agglutination.

We have had no spondylitis that I know of. We have had an incidence of some 70 per cent pruritus, which may be significant, since that is often a clinical finding in Hodgkin's disease.

Gordon's test has not been done. We have done animal inoculations but I can give you no results as yet.

I am sorry I am not prepared to answer the question about the mitoses in the cells. We have had numbers of cells which had very bizarre, piled-up nuclei, as though they had incompletely divided, but I can go no further than to make that statement.

(Dr. Carl V. Weller, Ann Arbor, Mich.) It might be a good plan to let some of these cases circulate through the Lymphatic Tumor Registry. I think they would be a very good addition to that series.

THE PATHOLOGY OF ENCEPHALITIS IN MAN CAUSED BY THE VIRUS OF THE EASTERN VARIETY OF EQUINE ENCEPHALOMYELITIS. Charles F. Branch and Sidney Farber, Boston, Mass.

Abstract. An outbreak of equine encephalitis in eastern Massachusetts in August and September, 1938, caused the death of over 90 per cent of 248 horses affected. Between August 15 and October 1, 1938, 26 human beings from 1 month to 60 years of age died of encephalitis. Seventy per cent of the patients were under 10 years of age. Studies by Webster and Wright, and Fothergill, Dingle, Farber and Connerley showed that the causative agent was the virus of the eastern variety of equine encephalitis. This report is based on portmortem examination of 17 patients who died in from 1 to 22 days after the onset.

Marked edema and congestion of the brain and cord, flattening of the convolutions, pressure cone formation, generalized congestion of the viscera and pulmonary edema were conspicuous gross findings. Microscopic examination revealed a severe diffuse meningoencephalitis, most marked in the basal ganglions and in the brain stem, and characterized by widespread nerve cell destruction and accumulations of inflammatory cells in perivascular spaces, the meninges, and in the numerous areas of necrosis. The cellular infiltration, largely neutrophilic in character in the early lesions, became predominantly large mononuclear and lymphocytic 6 to 20 days after the onset, a change which ran parallel to the cell types in the spinal fluid. Involvement of small vessels with neutrophilic infiltration and fibrin deposition throughout the walls was one of the most striking features of the disease. Demyelinization was found only where entire areas were destroyed in the inflammatory process. The cord was involved severely in only 1 case. No bacteria could be demonstrated in association with the early lesions. No inclusion bodies were found. The pathological picture simulates most closely that of St. Louis encephalitis.

Discussion

(Dr. Edwin F. Hirsch, Chicago, Ill.) I think an epidemic very much like this occurred last fall near Minot, North Dakota, and the lesions in the human brain were very much like those described by Dr. Branch. The material was tested at St. Louis to see whether the virus in this epidemic was like that of the St. Louis epidemic, but there was no confirmation. I wonder whether Dr. Clawson has anything to offer in that connection.

(Dr. Benjamin Clawson, Minneapolis, Minn.) No.

(Dr. Branch, closing.) As far as we know, this is the first demonstration of the eastern strain of equine encephalitis in the human being, and these I believe were adequately proved by Webster and Fothergill.

TRAUMATIC BRAIN STEM HEMORRHAGES. E. A. Linell, Toronto, Canada.

Abstract. Brain stem hemorrhages due to head injury can be divided into four groups in accordance with their anatomical situation. These situations are: (1) the subependymal tissues of the third and fourth ventricles; (2) the dorsal tissues of the upper midbrain; (3) the ventral tissues of the upper midbrain, involving the oculomotor nerve nuclei; and (4) the tissues of the lower midbrain and pons. In this series of 21 cases, 6 were subependymal, 8 were in the upper midbrain, and 7 were in the lower midbrain and pontine tissue.

In the first group the nerve cells in the hypothalamus and the floor of the fourth ventricle were involved in the hemorrhage. In 3 of these 6 cases there was a depressed fracture of the skull and in 1 of the remainder a gunshot wound of the frontal lobes was the cause of the hemorrhages.

The hemorrhages in the upper midbrain are mainly of interest from the damage which is necessarily caused to the oculomotor nerve nuclei, showing one of the mechanisms responsible for oculomotor nerve paralysis in cases of head injury. Some of these cases suggest bruising against the tentorium cerebelli as a possible mechanism for the midbrain hemorrhage.

The traumatic hemorrhages into the lower midbrain and pontine tissue are, as a rule, more massive than in the other groups. These massive hemorrhages, with the edema which accompanies them, are probably an important factor contributing to death. In the majority of cases in this group the patient died within a few hours after the injury.

A STUDY OF BRONCHIECTASIS IN FIFTY LOBECTOMY CASES. W. L. Robinson, Toronto, Canada.

Abstract. This represents a further study of the pathology of bronchiectasis based on fresh surgical material from 50 lobectomies for this disease. The findings more or less confirm those of our first presentation on 16 cases as reported in the *British Journal of Surgery*, 1933, **21**, No. 82.

In bronchiectasis the essential pathological process is a persistent infection of the bronchial wall leading to destruction of the musculoelastic elements and also, to a more or less degree, the cartilage plates. It is not an ulcerocavernous process as the other elements of the bronchial wall, cartilage plates, mucous glands and bronchial arteries are left intact. An occasional microscopic ulcer, however, may be found. The process might very well be compared to that of syphilis of the aorta. The inflammatory reaction in the subepithelial and muscular layers is constant in its presence and in the character of its wandering cell.

The problem of the etiology of this disease centers around the factors which make for the establishment and persistence of the infection. The infecting organisms are many and varied. The infection is apparently not specific in type, although the character of the inflammatory reaction suggests this to be so. The establishment of the infection is not as obscure as the reason for the persistence of the infection once established. Stagnation of the secretions in the lumen of the bronchus undoubtedly makes for persistence of the infection. This may be brought about mechanically by complete or partial occlusion of the proximal end of the tube by a tumor, foreign body, inflammatory mass, and so on. It may also be brought about

by any malfunctioning of the bronchial tubes such as paralysis of the ciliated epithelium whereby they are unable to clear the tubes properly of mucus and foreign material. Vascular sclerosis of the bronchial arteries may also play a part. Finally, in those cases where mechanical blocks can be eliminated it is suggested that there might be a physiological block occasioned by the presence of an area of metaplastic squamous epithelium at the proximal end of the tube. This would act as a barrier to the expulsion of mucus and débris by the ciliated epithelium in the lower part of the tube.

Ordinary respiratory movements of the bronchi are sufficient when the wall is diseased to produce the dilatations seen in these cases.

Discussion

(Dr. Max Pinner, Ithaca, N. Y.) I am interested in this paper, particularly in the statement that ulceration of the bronchial epithelium was found relatively rarely. From the cases I have studied I was under the impression that ulceration is a rather prominent feature and that large portions of the bronchiectatic cavity are involved. The bronchi are seen ulcerated throughout the entire wall, including all its structures, elastica, muscles and cartilage. Inflammatory infiltrations around the nerves in the bronchial wall are frequently seen. With this deep ulceration, which in the later stages is frequently partly replaced by intensive fibrosis, there occurs such a profound destruction of the middle sized and small bronchi that this is probably one of the very important causes of perpetuating the infection of the bronchi by preventing proper drainage. If one follows the bronchi in longitudinal sections one finds very frequently stenotic and dilated portions; in other words, bronchiectasis is frequently to a large extent bronchostenosis, and peripheral to the stenosis are seen bronchiectatic cavities.

The other point to which must undoubtedly be attributed the perpetuation of the infection is the almost constant simultaneous existence of upper respiratory infection, providing continuous infection of the lower portions. One remaining feature which should not be overlooked in bronchiectasis is the fact that one does not find bronchiectasis in an otherwise normal lung. The pulmonary parenchyma is always severely damaged, depending on the stage of the disease, by suppurating or organizing processes.

(Dr. William S. Stanbury, Hamilton, Canada.) During the past year I have studied 15 pneumonectomy and lobectomy specimens of bronchiectasis. Although our series of cases was of a younger age group, our findings are essentially the same as Dr. Robinson's. I think at this time, when there is an increasing tendency, particularly in the clinical literature, to emphasize extrabronchial factors in the etiology of bronchiectasis, Dr. Robinson's paper is very timely, demonstrating, as it does consistently, the essential lesion in the musculoelastic coat of the bronchus. In our series the majority of the cavities were lined by perfectly normal ciliated epithelium with well defined basement membrane; ulceration, when it occurred, was superficial, and where it occurred the underlying bronchial wall already had been destroyed extensively. I feel with Dr. Robinson that these lesions of the musculoelastic coat are fundamental to bronchiectasis, and ulceration when seen can be considered only an incidental finding. To the last speaker I would say we have seen equally good bronchiectatic lesions in the normal, collapsed, emphysematous and pneumonic lung. I do not feel an extrapulmonary process has any definite constant relation to the etiology of bronchiectasis.

(Dr. Robinson, closing.) I notice Dr. Pinner was talking about autopsy material. I found it rather difficult in going over a few autopsy specimens to be sure when one had ulceration, but I feel that in dealing with fresh material we are pretty safe. I am only reporting surgical specimens. We found very few ulcers and these were small and superficial. There is nothing in our series to indicate that the process is an ulcerocavernous one. I am convinced of that, but I cannot argue further as I have not gone over any extensive autopsy series. This is purely a group of surgical cases. As to the suggestion about the persistence of infection, I am ready to accept that. I feel, however, there is something local, some change in the local condition which makes for persistence of the infection in the bronchi, rather than a general inflammatory reaction in other parts of the respiratory tract.

I am glad to find that Dr. Stanbury corroborates our findings.

AGE AND SITE OF OPERATION IN RELATION TO POSTOPERATIVE PULMONARY EMBOLISM. J. S. McCartney, Jr., Minneapolis, Minn.

Abstract. It is commonly stated that operations on the lower part of the abdomen are often followed by pulmonary embolism. This is true because of the age at which such operations are done. If operations on various parts of the body were uniformly distributed throughout all decades, pulmonary embolism would not appear to be most common after lower abdominal operations. To prove this statement large numbers of operations from a variety of sources were compiled according to age and the part of the body operated upon. This compilation showed that the majority of operations on the head and neck and appendectomies are done before the age of 30 years, and this explains why such operations are only occasionally followed by embolism. Herniorrhaphy is not infrequently followed by embolism, about one-half of such operations being done after the age of 30 years, when practically all the instances of embolism appear. The majority of biliary and abdominal gynecological operations are done after the age of 30 years and so, not rarely, embolism takes place. Suprapubic bladder and prostate operations are done only very rarely before the age of 50 years and as a result pulmonary embolism is quite frequent after such operations. Operations on the extremities are carried out rather uniformly through all decades and embolism occurs in all decades, but particularly so in the older age periods.

From a study of the postmortem records of 3661 operative deaths it was found that although fatal pulmonary embolism may occur at any age, its frequency increases with age. The postmortem records were not from the operations used in determining the time of life at which operations are done. The conclusion was reached that it is not so much the part of the body on which operations are performed that is the cause of the embolism as it is the age of the patients at the time operations are carried out.

Discussion

(Dr. Edwin F. Hirsch, Chicago, Ill.) I should like to ask if the source of the emboli was traced in this series of reports, and whether the upper extremities were as common a site of origin as the lower extremities.

(Dr. Kornel Terplan, Buffalo, N. Y.) I should like to know whether in Dr. McCartney's material there was any evidence of fatal postoperative thrombo-embolism following operations on the brain.

(Dr. Carl V. Weller, Ann Arbor, Mich.) My own impression of this material is that we have been shown simply that the emboli we know about are those which occur in individuals who have impaired circulation. Probably emboli are equally common at all ages, but only those which occur in individuals who have an impaired circulation become known to the pathologist. If you had substituted "fatal embolism" for the word "embolism" in your conclusions I would have been in full accord with you.

(Dr. McCartney.) We traced as far as possible the source of each embolism. We have a few instances of fatal embolism following operations on the lower extremities and some on the upper extremities. As is commonly the case in our embolisms, the site is in the lower extremities or in the pelvis. In only about two-thirds of the instances were we able to find the source because we could not do a sufficiently thorough dissection most of the time to find it.

In answer to Dr. Terplan, we have a few instances of embolism following operations on the head. We do not get many deaths following surgical procedures on the head, or at least, we do not get many postmortems.

I should have stated I was going to discuss fatal embolisms. I left out the non-fatal embolisms intentionally. If we consider the non-fatal embolisms then the figures are practically trebled. If we took all infarctions or non-fatal embolisms, it comes back to the question as to what percentage of embolisms of the lung are fatal. There are statements of anywhere from 10 per cent up.

(Dr. Weller.) Do you believe that embolism can occur in the lungs without infarction in individuals who have a normal circulation?

(Dr. McCartney.) I am quite sure of it.

(Dr. Weller.) So that if you took the non-fatal emboli you still would not have an equal quota of them present in the earlier years?

(Dr. McCartney.) Yes.

(Dr. Harry C. Schmeisser, Memphis, Tenn.) How large must an obstructed artery be for a pulmonary embolism to be fatal?

(Dr. McCartney.) That is a question because it involves a number of things — the presence or absence of previous pulmonary disease and the presence or absence of pneumonia. The common statement in the literature is that in animals it requires somewhere around 60 to 65 per cent occlusion of the pulmonary artery before death can result, but as I see it in animals, most of the investigators were dealing with young animals and not with animals of an age comparable to the human being, and they have presumably a sound cardiovascular system. We get fatal emboli from occlusion of one lung. Virchow recognized that. He also recognized that we can get death, perhaps not really sudden, but death, after a time (minutes or hours or days) from a shower of small emboli, and with these showers of small emboli we occasionally have infarction, whereas with the massive embolisms which occlude the branches of the pulmonary artery we do not have infarction. There is no time for an infarct to develop and it does take a little time to form.

(Dr. Terplan.) Did I understand that Dr. McCartney found the source of the fatal thrombo-embolism in all cases?

(Dr. McCartney.) In two-thirds of them.

(Dr. Terplan.) We found it rather difficult to be certain as to the sources of the massive thrombo-emboli occluding the main stem of the pulmonary

artery. Even most careful dissection of the peripheral veins not infrequently failed in our experience to detect a thrombus. Nor could we find any endothelial damage in the larger veins of the lower extremities which might have pointed to the site of detachment of the massive thrombus. To me, the sudden fulminating fatal thrombo-embolism appears quite problematic. May I ask Dr. McCartney what his opinion is as to the theory of Havlicek? In our more limited experience we still find that sudden fatal surgical pulmonary embolism occurs almost exclusively following operations within or near the abdominal cavity.

(Dr. McCartney.) I have read Havlicek's paper. It sounds too simple. The thing that comes up in that connection is that one does not expect to get thrombosis in individuals who are jaundiced, because the jaundiced individual is supposed to bleed, instead of develop thromboses, and by and large that is true, but we not infrequently get fatal and non-fatal embolism in individuals who are jaundiced. It is rare, but it does occur. I think undue emphasis has been placed on the pelvic and femoral veins as a source for embolism. There is considerable information in the literature that the primary thrombus is low down in the extremity, in the ankle or foot, and can propagate into the femoral and iliac veins. We may milk out the large clots, if we cannot dissect them, but the small primary thrombi in the legs we do not get. We cannot carry out a sufficient dissection to get at them.

THE RELATION OF THE INSULIN HYPOGLYCEMIC REACTION TO SHOCK. Warren C. Corwin (by invitation), Philadelphia, Pa.

Abstract. It was proposed to ascertain the relation of the insulin hypoglycemic reaction to shock by determining the presence or absence of hemoconcentration during "insulin shock" and by noting changes present in the viscera after death from large doses of insulin. Determinations of the hemoglobin content and the number and volume of erythrocytes in the blood of dogs and rabbits indicated that hemoconcentration does not occur incident to hypoglycemic reactions resulting from injections of non-fatal or fatal doses of insulin. Visceral evidences of capillovenous congestion and increased capillary permeability were not seen in animals after death by insulin hypoglycemia. The mechanism of death resulting from large doses of insulin is not the same as that of shock. The term "insulin shock" is confusing and should be abandoned.

Discussion

(Dr. Joseph Tannenberg, Albany, N. Y.) I agree entirely with Dr. Corwin that insulin shock is quite different from common shock or wound shock by its pathological manifestations. Therefore, it might perhaps be wise to agree on another name for it. In experimental insulin shock the animals die of lesions in the brain with large areas of cortical cells being bleached out, and ganglion cells in other parts of the brain, especially in the medulla, being similarly affected. The lesions of the central nervous system are so predominant that they obviously are the cause of death. When rabbits were seized by convulsions during insulin shock I have, however, also observed vascular reactions which might be of some significance. In such cases the ear arteries became so constricted that it was frequently impossible to obtain

blood from the ear veins during or some time following a seizure, even when local irritants such as xylol were employed.

(Dr. Corwin.) I agree with Dr. Tannenberg. I should have emphasized that the term "insulin shock" is after all confusing and therefore should no longer be employed. Some such term as "insulin reaction" would be preferable. I also agree that the mechanism of death from large doses of insulin is primarily a cerebral one. It is undoubtedly due to the inability of the brain to utilize oxygen in the presence of a reduced glucose content of the blood. Recently studies on the protein content and osmotic pressure of the serum in man and the dog during insulin hypoglycemia have been published by Butt and Keys. These authors likewise concluded that so-called insulin shock bears no close relation to other types of shock.

STUDIES ON METAPLASTIC OSSIFICATION. Sheldon A. Jacobson, Brooklyn, N. Y.

Abstract. It has been demonstrated by Huggins and others that the transplantation of urinary epithelium will provoke metaplastic bone formation in the rectus muscle of the dog. By experiments on the rat it was found to be refractory to this procedure. In an attempt to change the animal's reaction, 170 experiments on 102 animals were performed as follows:

Bladder segments, dome of the bladder and minced bladder were transplanted into the muscle peritoneal sac, spleen, liver and kidney. Bladder transplants with: (*a*) subcutaneous injections of calcium chloride in the graft area; (*b*) calcium lactate 0.5 per cent as drinking water; (*c*) a 50 x normal therapeutic dose of viosterol; (*d*) a 500 x normal therapeutic dose of viosterol; (*e*) 0.025 mg. elementary phosphorus daily; (*f*) 0.05 mg. elementary phosphorus daily; (*g*) the same after destruction of one kidney by radium emanation seed; (*h*) implantation in the same area of boiled beef bone, boiled rat bone and 50 x normal viosterol and silver nitrate crystals were all tried.

Kidney segment, kidney cortex, kidney medulla, kidney pelvis and ureter transplants were made in muscle.

Boiled rat bone, boiled beef bone and silver nitrate crystals were also implanted.

The vessels of one kidney were ligated.

Removal, eversion and replacement of the dome of the bladder were performed.

Removal of the dome of the bladder and replacement by fascia were also done. Removal, eversion and replacement of the dome of the bladder and administration of elementary phosphorus were done. Bladder transplants were made in pregnant females.

These attempts were almost uniformly unsuccessful, there having been a very feeble bone formation in 5 scattered experiments.

The guinea pig was studied and was found to produce bone almost uniformly on transplantation of bladder tissue to muscle. In an attempt to ascertain whether the calcification was primary and acted as a stimulant to ossification, or whether the deposition of bone matrix was the first step in the process, an attempt was made to change the reaction of the guinea pig in this respect by the administration of a scorbutogenic diet. In no case was bone deposited nor was calcification observed.

Despite the refractoriness of this animal to rickets, the experiment was re-

peated with the animals fed a rachitogenic diet. Two out of 10 animals showed bone formation.

On the basis of histological pictures found incidentally in these experiments the theory of the histogenesis of osteochondroma and of the epiphyseal plate is suggested.

Discussion

(Dr. Edwin F. Hirsch, Chicago, Ill.) I should like to ask if any appreciable amount of cartilage was found. I understand in the experiments which Huggins performed that there was no appreciable amount of cartilage associated with bone formation.

(Dr. Jacobson.) In the case of the dogs there was a large amount of cartilage in more than 1 animal. The slide I showed was not an incidental finding. I have a couple more slides but there was no point in showing them as they add nothing. I did not find cartilage in the guinea pigs.

THE CAUSAL SIGNIFICANCE OF TRAUMATIC OSSIFICATION OF THE FIBROCARTILAGE IN TENDON INSERTIONS. Edwin F. Hirsch and (by invitation) Russell H. Morgan, Chicago, Ill.

Abstract. The early stages of the lesion (11 cases) of traumatic ossification contain large amounts of fibrocartilage or hyaline cartilage continuous with bone in varying degrees of differentiation. Some portions seem to be ossifying cartilage, others have lamellar trabeculae containing residues of cartilage. The late stages of the lesion have a high content of lamellar bone and only small traces of cartilage. These conditions imply the origin of bone in a cartilage matrix that is endochondral bone formation.

Fibrocartilage is a normal constituent of the insertions of many tendons (69 from postmortem material) in which traumatic ossification occurs. A reactive or reparative growth of these tissues initiated by trauma provides a simple explanation for the lesion of traumatic ossification.

POLYVINYL ALCOHOL STORAGE DISEASE. W. C. Hueper, New York City.

Abstract. Polyvinyl alcohol is one of several plastic substances developed during recent years by the chemical industry, and is used in the manufacture of resins, lacquers, and so on. Polyvinyl alcohol, introduced as an aqueous colloidal solution subcutaneously or intravenously into rats and rabbits, respectively, is retained in the organism to an appreciable extent and for a considerable time at the site of injection, as well as in numerous remote organs. The injected chemical is stored (*a*) in the reticuloendothelial cells of the spleen, liver, suprarenals and lymph nodes; (*b*) in the endothelial cells of the blood vessels of the brain, lung and kidney (glomeruli); and (*c*) in histiocytes of various organs (lungs, chorioid plexus, testes, retroperitoneal tissue), in fat cells, and in ganglion cells and glia cells of the brain. The inner wall of blood vessels often is covered by a coat of polyvinyl alcohol which may result, especially in the lung, in the production of endothelial swelling and subsequent cellular damage characterized by the presence of foreign body giant cells and phagocytic foam cells containing polyvinyl alcohol. The polyvinyl alcohol present in tissues and cells can be demonstrated readily in

sections by its characteristic and specific blue color reaction with Lugol's solution. The character and extent of the organic lesions produced are related to the physicochemical properties peculiar to polyvinyl alcohol (large molecular size, viscosity of the aqueous solution, precipitability from a liquid, colloidal state to a particulate, solid state by changes of the salt concentration of the medium, tendency to form films, and marked resistance to chemical as well as enzymatic, metabolic degradation). Studies with polyvinyl alcohol may provide a method by which additional information may be obtained possibly in regard to the physicochemical action-mechanism of certain biologically important, macromolecular substances or aggregates (proteins, polysaccharides, lipids) under normal and pathological conditions (immunity reactions, malignant tumors, storage diseases, degenerative lipoidoses).

Discussion

(Dr. Herbert S. Reichle, Cleveland, Ohio.) I thought that hitherto the endothelial cells of the larger vessels have not been regarded as a part of the reticuloendothelial system in the sense that they would be phagocytic. I noticed that in the vein of the lung the endothelium contained polyvinyl alcohol. Are you sure in this case there were not thrombi, either mural or agglutinative, and that the cells in these contained the polyvinyl alcohol?

There is a further point about the brain — the remarkable thing that the ganglion cells should contain this material — and the question arises whether this is a lipoidhistiocytosis in the sense of the words hitherto used, or whether this material is taken up by cells in general due to some metabolic change which we cannot define more closely.

(Dr. Carl V. Weller, Ann Arbor, Mich.) It seems to me the occurrence of this material in ganglion cells and glial cells, as well as in the histiocytes, is one of the most important things about this presentation because it brings to mind Tay-Sachs disease in which this distribution occurs.

(Dr. Hueper, closing.) I intended to take up the subject of phagocytosis in my paper, but on account of the lack of time I was prevented from doing so. I may therefore read the passages referring to this subject contained in my paper:

"The retained portion of the polyvinyl alcohol is stored in reticuloendothelial cells, endothelial cells, histiocytes, glia cells, and various other cellular elements which are ordinarily not included among the cells possessing phagocytic qualities. These are fat cells, fibroblasts, endothelial cells of larger vessels, ganglion cells, and so on. On the other hand, leukocytes, which are credited usually with pronounced phagocytic qualities, do not ingest this chemical in spite of an intimate contact with it. Mononuclear cells, however, may form an exception. Phagocytosis of liquid foreign matter does not seem to follow the same rule as to cellular types participating in this phenomenon as that established for the phagocytosis of solid particulate matter. The physicochemical properties of the particular substance involved determine apparently the types of cells which may take a part in phagocytic activities."

There can be no doubt that endothelial cells of larger vessels phagocytize polyvinyl alcohol, as this substance can be demonstrated in these cells by the specific reaction with Lugol's solution.

MUSCULAR DYSTROPHY IN BILIARY FISTULA DOGS. E. D. Warner and K. M. Brinkhous (by invitation), Iowa City, Ia.

Abstract. Progressive degeneration of skeletal muscles resulting in extreme paresis occurred frequently in a group of dogs with biliary fistulas of the gall bladder-renal type. The distribution and type of lesions of the muscle observed are comparable to those of the "nutritional muscular dystrophy" in rabbits and guinea pigs described by Goettsch and Pappenheimer. One sees Zenker's degeneration with connective tissue replacement of the necrotic muscle cells and marked atrophy of fibers which do not undergo necrosis. No lesions have been found in the nervous system. The disorder is thought to result from a dietary deficiency incident to the absence of bile in the intestine. The animals were fed a mixed diet which has been entirely adequate for animals not having biliary fistulas. Simple inanition can be excluded as the cause of this condition.

ACUTE HYPERTROPHIC HEPATITIS. J. D. Kirshbaum and (by invitation) H. P. Popper, Chicago, Ill.

Abstract. Fifteen cases of acute primary parenchymatous jaundice are described. Clinically they were characterized by a fulminating fatal course, death usually occurring within 10 days, and only 2 cases showed a subacute course of 1 to 3 months duration, but without any signs of healing. The onset with severe jaundice was frequently accompanied by fever, chills, gastro-intestinal signs, pains in the muscles and joints, and leukocytosis. Later there were cerebral manifestations associated with nitrogen retention and anuria. In the etiology drug poisoning was excluded, but there were mentioned in the history food poisoning, upper respiratory infections, gonorrhea, and in 1 case *Paratyphoid B* was isolated. Clinically a diagnosis of hepatitis was suggested in some cases. Despite the similarity of the clinical picture to acute yellow atrophy of the liver, it was differentiated by the marked enlargement of the liver, as seen in catarrhal jaundice. At autopsy the large liver (average weight 2150 gm.) was striking. The spleen was enlarged with increased fibrosis, congestion and focal aggregations of erythrocytes simulating hemorrhages. The kidneys were swollen and showed microscopically degenerative signs. Histologically the liver showed: first, damage to the cells with necrosis and necrobiosis, indicated by disappearance of liver cells, presence of granular débris, bile storage in the cells, collapse of the framework, and also fatty changes. The changes were mostly localized in the center of the lobules. Second, a marked toxic edema was visible with enlargement of the spaces between the blood capillaries and the liver cell cords in which coagulated plasma proteins could be demonstrated. Various degrees of such a serous hepatitis (Roessle, Eppinger, Popper) due to damage of the blood capillaries of the organ produced a disturbance of the structure of the cell cords with segregation of single or groups of cells — so-called dissociation. That the dissociation is not a primary parenchyma cell process is revealed by the intact nuclear staining of the round shaped dissociated cells. Sometimes the parenchyma cells are entirely washed away and appear in the lumen of the veins. Attempts at healing with regeneration of liver cells and proliferation of bile ducts were seen in Case 16. Jaundice appeared subsequent to an alimentary intoxication which, however, decreased. Death was due to an

ulcerative colitis. Thus, we are dealing with a transitional stage between catarrhal jaundice and acute atrophy of the liver with damage to the parenchymatous cells and the capillaries. The latter in the form of a serous hepatitis causes the enlargement of the organ, and the same explanation may be valid for the enlargement of the liver in catarrhal jaundice. The cause of the icterus is a combination of a localized breaking up of the liver cell cords due to necrosis or dissociation with a consequent communication between bile capillaries and tissue spaces, and a generalized functional damage is produced by the serous hepatitis. The latter prevents the excretion of the regurgitated bile occurring in necrosis alone. This explains all forms of parenchymatous jaundice. The presence of the toxic edema would indicate active therapy for dehydration of the liver by means of intravenous injections of hypertonic sugar solutions in all types of parenchymatous hepatitis.

Discussion

(Dr. Carl V. Weller, Ann Arbor, Mich.) This is a type of case that bothers us from time to time, particularly from the standpoint of etiology. I always suspected there were unknown toxic factors rather prominent in this group. Often, particularly in children, we have been unable to determine what the toxic factor might be, in spite of efforts at detective work.

(Dr. Kirshbaum.) The child presented showed *B. typhosus* bacteriologically. That was the only case in which we were able to identify organisms.

THE DEVELOPMENT OF HEPATIC CIRRHOSIS FOLLOWING HYPOPHYSECTOMY. Irvine H. Page (by invitation), Indianapolis, Ind., Irving Graef, New York City, and (by invitation) Joshua E. Sweet, New York City.

Abstract. During observations of the effect of complete hypophysectomy on the induction of hypertension by renal ischemia (after Goldblatt), 2 dogs (male and female) were kept alive 2½ years. They developed diabetes insipidus, marked adiposity, loss of gonadal function, and changes in their coats. At postmortem examination nodular hepatic cirrhosis was encountered and cortical atrophy of the adrenal glands, as well as involutional changes in the gonads.

Three additional hypophysectomized dogs (furnished by Dr. Bodo), with a similar clinical syndrome and surviving 9 months to 1 year, exhibited milder grades of portal cirrhosis. Fatty change was present in all but was not remarkable.

The endocrine relation of the liver to the pancreas is well known, but a relation to the pituitary gland and its nervous connections is not well established. In human pathology, Wilson's disease, the occurrence of gynecomastia, and loss of hair in cirrhosis of the liver, are findings which imply a relation that may be reciprocal.

VITAMIN A STORAGE IN ACTIVE AND ARRESTED CIRRHOSIS. Alvin J. Cox, San Francisco, Cal.

Abstract. In 43 fatal cases of cirrhosis of the liver the vitamin A content of the liver, as measured by the antimony trichloride reaction, was studied in relation to the clinical course of the disease and the character of the lesions in the liver. The livers in which the pathological process showed the greatest

activity contained practically no vitamin A, whereas when the lesions showed little or no evidence of activity the stored vitamin A approached the average normal value.

Discussion

(Dr. James S. McCartney, Minneapolis, Minn.) I should like to know how many of these cases were patients who died of cirrhosis — whether any of these were the so-called accidental findings at postmortem, and whether Dr. Cox found, if they fell into those two groups, degenerative changes, or, as he spoke of it, signs of activity in the livers of individuals which have cirrhosis as an accidental finding.

(Dr. Carl V. Weller, Ann Arbor, Mich.) I wonder whether the use of the term "precirrhotic" for the degenerative changes here would not have made the presentation a little clearer. I think of cirrhosis as beginning with fibrosis, so that it seems to me that if you had said precirrhotic and cirrhotic activity I would have understood it a little better.

(Dr. Cox.) In answer to Dr. McCartney's question, I am sorry I have not prepared a table correlating symptoms of cirrhosis and vitamin A content of the livers. In some of the cases cirrhosis was not a cause of death. There was good correlation between the clinical and the anatomical pictures. Most of those cases with death from esophageal hemorrhage or intercurrent disease, or without the clinician having been aware of the cirrhosis, fell into the group of arrested cirrhosis and showed little evidence of degeneration of liver cells.

EXPERIMENTAL TROPICAL CIRRHOSIS. Philipp Rezek (by invitation), Miami, Florida.

Abstract. Experimental studies on tropical cirrhosis are reported. The peculiar so-called Indian infantile cirrhosis is described. Different forms of hepatitis were produced by feeding dogs with the hot spices that constitute part of the daily diet in British India.

Alterations in the livers of newborn puppies from females who had been fed with these Indian hot spices during pregnancy and lactation were also found. These alterations are similar to those found in newborn infants in India suffering and dying at the rate of 15,000 to 20,000 annually from this type of cirrhosis. It is believed that there is a field for tropical research in the study of hot spices in relation to the intra-uterine effects on the newborn and effects during lactation.

It is also believed that further chemical analyses of condiments would be of help, since epilepsia of the human type in dogs, after administration of pyrol, a substance contained in pepper, was produced.

READ BY TITLE

REPORT OF A FATAL CASE OF HISTOPLASMOSIS IN INFANCY. Arthur L. Amolsch, Detroit, Mich.

THE SPECIFICITY OF THE HUMAN INFLUENZA VIRUS FOR PULMONARY TISSUE, AS SHOWN BY EXPERIMENTS ON MAMMALIAL FETUSES. N. Paul Hudson and (by invitation) Oram C. Woolpert and Herman A. Dettwiler, Columbus, Ohio.

EXPERIMENTAL LESIONS OF ARTERIES WITH HUMAN FAT, FATTY ACIDS, SOAPS AND CHOLESTEROL. Oscar O. Christianson (by invitation), Tuscaloosa, Ala.

THE ETIOLOGY OF CONGENITAL BILATERAL POLYCYSTIC KIDNEYS. James E. Davis, Ann Arbor, Mich.

REFRACTORY ANEMIA PRODUCED BY DIFFUSE PLASMOCYTOMAS. Walter W. Jetter, Buffalo, N. Y.

THE INFLUENCE OF CUTANEOUS INFLAMMATION ON THE ACTION OF STAPHYLOCOCCUS TOXIN. Harold B. Kenton, Chicago, Ill.

CYSTS OF THE THIRD VENTRICLE. E. A. Linell, Toronto, Canada.

RUPTURED CONGENITAL ANEURYSM AS A CAUSE OF INTRACEREBRAL HEMORRHAGE. E. A. Linell, Toronto, Canada.

LESIONS OF THE MYOCARDIUM ASSOCIATED WITH A LOW POSTASSIUM DIET. Ernst Mylon (by invitation), R. M. Thomas and M. C. Winternitz, New Haven, Conn.

Abstract. White rats, fed a diet which limited the potassium intake to 1.2 to 1.5 mg. per day, lost weight steadily and lived on this diet for an average of 29 days. Control rats on the same basal diet with added potassium chloride (12 mg. per day) increased in weight during the period of the experiment and seemed healthy in every respect.

The only lesions of significance found in the animals fed low potassium diets were confined to the myocardium. This showed focal and diffuse areas of necrosis with replacement of the muscle fibers by young cellular connective tissue. The lesions, when extensive, were chiefly subendocardial and not infrequently complicated by mural thrombi.

ACTIVE ENDOCARDITIS IN THE NEWBORN (REPORT OF 2 CASES). Alfred Plaut, New York City.

Abstract. In a previous paper (*Arch. Path.*, 1935, **20**, 582) we were able to record what we considered the first microscopic evidence of active endocarditis in the newborn. A second instance of this probably extremely rare condition has come to our attention.

On the tricuspid valve of a 1 day old male baby, who died of a subarachnoid hemorrhage, generalized hyperemia of the brain, suprarenal hemorrhage and atelectasis of the lungs, was a small, brittle, grayish cluster, 5 by 2 mm., attached to the edge of the anterior leaflet and surrounding the contiguous chordae tendineae. There were no other unusual lesions in the heart. Two pin-point blood cysts (so-called valvular hematomas) were seen in the mitral valve.

A search was made for foci of infection in the organs. A few small vessels in the kidneys were found distended with fibrin-like masses and a few leukocytes. Numerous small vessels in the lungs, some of them definitely of arterial character, contained masses similar to those in the kidney. Occasionally there was a slight overgrowth of the endothelium of these vessels. In wide thin walled veins at the lateral edge of the thyroid gland older thrombotic masses

were found consisting of rather compact fibrinoid material and numerous, partly mononuclear partly polymorphonuclear cells. At two points the thrombotic mass was found attached to the vessel wall. A thick walled vessel in a villus of the placenta also contained a fibrinous mass with a few leukocytes. The hemorrhages which were found in different organs, including the subepicardial tissue, consisted almost exclusively of red cells, in marked contrast to the intravascular masses containing numerous leukocytes.

The microscopic picture of the endocarditic lesion was somewhat different from that in our 1st case. The endocarditic lesion consisted mainly of fibrin which at several points was adherent to the valve, the superficial layers of which were ulcerated at these points. Leukocytes were scarce in the fibrinous mass, but the loose hemorrhagic masses surrounding it contained many. The nuclei in the valve near the endocarditic lesion were numerous and swollen. No leukocytes were seen in the valve itself. No microorganisms were found in crush smears of the vegetations and in the paraffin sections. Aerobic and anaerobic cultures of the spleen remained sterile.

As in our previous case, the pathogenesis of the endocarditis remained obscure. But while in the 1st case no other inflammatory lesion could be found, there was in the 2nd case evidence of inflammation. The thrombus in the neck vein obviously was not recent. The thrombotic masses in the lungs and kidneys appeared to be more recent, but the endothelial overgrowth in vessels of the lung also denoted a certain duration. Unfortunately there is no record of the condition of the middle ears.

No infectious disease of the mother during pregnancy was noted. She had high blood pressure and edema of the feet. The membranes ruptured 24 hours before onset of labor.

The fact that the mother had no symptoms of infectious disease during pregnancy is not astonishing, since the mother may remain in good health even in true sepsis of the fetus (Wohlwill and Bock, *Arch. f. Gynäk.*, 1928, **135**, 271, Case 3). The possibility of an infectious focus in the neck or the head should be considered on account of the adherent thrombus in the neck vein. The most common inflammatory process in a newborn we might think of in this connection would be otitis media. In the cases reported by Hemsath (*Arch. Otolaryng.*, 1936, **23**, 78), however, no mention is made of evidence of inflammation in other parts of the body with the exception of so-called pneumonia of the newborn. We have examined the organs of 36 newborns in an attempt to find similar intravascular thrombotic and leukocytic masses but with negative results.

These 2 cases of active endocarditis in an otherwise normal heart, on normally formed valves, represent something different from the postulated endocarditic processes whose sequelae are found on malformed valves. What would become of such an endocarditis in case the infant survived remains doubtful. The case of Püschel (*Arch. f. Kinderh.*, 1938, **114**, 1) seems to indicate that such an endocarditis might heal without major defects in the valve.

A Rare Case of Neurofibroma of the Mesentery Resected During the Latter Months of Pregnancy Followed by Uneventful Recovery and Normal Delivery. S. H. Polayes and (by invitation) J. A. Timm, Brooklyn, N. Y.

Abstract. A colored female, aged 20 years, para I, gravida II, and 6 months pregnant, was admitted to the Cumberland Hospital on Oct. 24, 1938. For about 1 month prior to admission she had been conscious of an enlarging epigastric mass above the womb. This was accompanied by attacks of abdominal pain, backache, vaginal discharge and bleeding.

Neither the past nor the family history was remarkable. Physical examination revealed small nodules deep in the skin of the neck and abdominal wall, and a non-tender, freely movable, large, firm, ovoid mass situated in the mid-upper abdominal region. A fetus of about 6 months gestation was visualized in utero by x-ray examination. When a miscarriage appeared imminent a laparotomy was performed and a large segment of ileum and a tumor of its mesentery were removed. A side-to-side anastomosis of the remaining ileum was then performed. The patient made an uneventful recovery and later was delivered of a full term normal child.

A summary of the pathological report of the tissue removed at operation is as follows:

The specimen is a coil of ileum, 60 cm. long; the wall and mucosa are thickened; the mesentery is 8 cm. thick and composed of discrete, pearl gray, glistening firm nodules, some of which are cystic and discolored red and blue by hemorrhage. Microscopic examination of the specimen reveals numerous groups of ectopic sympathetic neurons lying immediately beneath the lining mucosal epithelium, the latter being in areas composed mainly of goblet cells. The submucosa also contains these neurons and in addition numerous eosinophils. The muscularis is thickened by bundles of dense fibrous tissue which are continuous with the mesenteric nodules. The latter are composed of neurofibromatous structures, some also presenting myxomatous changes. Definite neurons are not demonstrable in the nodules of the mesentery.

About 1 week postpartum one of the subcutaneous nodules from the neck was removed for examination. This revealed a neurofibroma identical in structure with the neurofibromas of the mesentery. In one of these nodules the nerve fibers constituting the neoplasm were continuous with those of a normal nerve trunk in the periphery of the mass.

The patient had a normal puerperium and was discharged from the hospital in good condition without any undue symptoms. When last seen (about 2 months later) she was in a normal state of health and the subcutaneous nodules in the neck and abdominal wall were found to be greatly reduced in size.

The Demonstration of Plasma Proteins in the Tissue by means of Fluorescence Microscopy. Hans P. Popper (by invitation), Chicago, Ill.

Abstract. The escape of plasma proteins into the interstitial tissue caused by damage to the capillaries and loss of semipermeability, with subsequent edema (serous inflammation), may occur in the loose interstitial tissue of the large parenchymatous organs. It produces malnutrition of the paren-

chymatous cells, disturbances of the structure, and opens the pathway for toxins in the tissue; if progressive, it is followed by sclerosis. The histological demonstration of serous inflammation meets with the difficulty that the plasma proteins show no microscopic differences from the tissue proteins. The observation of the spontaneous fluorescence of unstained slides of normally embedded organs, fixed in Carnoy's solution, reveals a difference between both types of proteins. The plasma proteins within the blood vessels and those escaping into the interstitial tissue are clotted and granular, and show a brown fluorescence as distinguished from the gray to blue of the tissue proteins. By staining with fluorescent dyes (fluorochromy) the difference can be exaggerated. With methyl green and thioflavine S at pH 6.0 the plasma proteins become a deeper brown color.

The serous hepatitis, as seen in coma, intoxications, infections, and especially in the first stage of parenchymatous jaundice, is characterized by wide spaces between the capillary wall and the liver cell cords which are filled with brown plasma proteins. This proves that the Disse space is not an artificial postmortem product due to shrinking or autolysis. The capillary wall is seen more distinctly in fluorescence than in the usual light. The wall of the capillaries is thicker in serous inflammation and is a sign of capillary involvement in contrast to the increased permeability. The thickening is probably due to an edema of the wall and may be followed by sclerosis of the capillaries with complete loss of permeability.

Serous myocarditis may be seen in all forms of suffocation, intoxication, and infection, but especially in acute rheumatic myocarditis and a hypertensive heart. The capillaries reveal a thickening of the wall and are surrounded by protein granules. In hypertensive heart disease where these changes are extensive, proliferation of connective tissue fibers in the edematous interstitium is visible (myocarditis serofibrosa), with consecutive scar formation due to capillary changes.

The slightest degree of serous nephritis is characterized by an escape of plasma proteins through the filtering glomerular loops (albuminuria), often combined with thickening of the wall of the loops (as in nephrosis). In the tubules the plasma proteins may be mixed with those originating from the tubular cell plasma. The next stage is the escape of proteins in the area of the reabsorbing capillary aggregations in the renal medulla. The proteins are visible between the capillaries, as seen in disturbances of bloodflow, hypertension and pyelonephritis. In the chronic stage proliferation of the connective tissue is seen as in chronic pyelonephritis, diabetes and cirrhosis of the liver. The severest stage of serous nephritis is the generalized edema of the kidney with the presence of serum proteins between the capillaries and the tubules of the cortex also. This may occur in acute glomerulonephritis and severe infections, sometimes combined with severe functional damage. It is the prodromal stage of the proliferation of connective tissue in the interstitium as seen in chronic nephritis or malignant hypertension.

TUMORS OF THE SYMPATHETIC NERVOUS SYSTEM. Edith L. Potter and John M. Parrish (by invitation), Chicago, Ill.

Abstract. Neuroblastoma and ganglioneuroma are universally conceded to be tumors composed of cells representing different degrees in the development of primitive tissue arising originally from the neural crest of the em-

bryo. The structure varies from tumors composed entirely of completely undifferentiated cells (sympathogonia), moderately differentiated (sympathoblasts), or completely differentiated (ganglion cells) to those in which there is a mixture of all elements. Schwannomas have been found in association with chromaffin tumors, but only rarely in a patient with a neuroblastoma or a ganglioneuroma.

The case reported is that of an infant born dead 2 months prematurely in whom multiple tumors were present. The entire chain of paravertebral sympathetic ganglia was transformed into a continuous mass of tumor tissue 1 to 1.5 cm. in diameter. Fused with the inferior surface of each adrenal were lobulated circumscribed tumors, each measuring 5 by 5 by 6 cm. Connecting these across the midline was a similar tumor of approximately equal size. The wall of the urinary bladder was composed of firmer, more fibrous tumor tissue which measured 3 cm. in thickness at its greatest width. It extended posteriorly and encircled the rectum, the fibers ending at the anus. Numerous, small encapsulated masses of tumor tissue were present throughout the posterior part of the abdominal cavity. There was an extreme hypertrophy of all nerves in the body, particularly those of sympathetic origin. Multiple minute tumor nodules were present throughout the liver.

Microscopically all of the tumors show a general similarity in structure and are composed of sympathogonia, sympathoblasts, ganglion cell and nerve fibers, with irregular areas of hemorrhage, calcification and necrosis. Sympathagonia form the main portion of the adrenal tumors; ganglion cells and fibers compose the greater part of the ganglion tumors, but all types form a diffuse mixture in all tumors.

The tumor of the bladder is different from those in other locations. It consists of large masses of tissue composed of elongated nuclei and fibrillar material separated into bundles by collagen fibers. In one area groups of immature ganglion cells are present, but the greater part of the tumor consists only of Schwann cells and fibers and is characteristic of a Schwannoma.

The hypertrophy of the nerves is due to an increase in Schwann cells accompanied by local areas of excessive acellular fibril proliferation.

This case exemplifies the interrelation of neuroblastoma, ganglioneuroma and Schwannoma. The tumor is not due to activation of misplaced rests of embryonic tissue but to some condition affecting the entire sympathetic nervous system, stimulating generalized neoplastic development.

OBSERVATIONS ON THE VIRULENCE AND OTHER PROPERTIES OF STREPTOCOCCI ISOLATED IN STUDIES OF INFLUENZA. E. C. Rosenow, Rochester, Minn.

Abstract. By the use of dextrose brain broth and soft dextrose brain agar it has been possible to isolate a streptococcus from the nasopharynx in all and from the blood in about one-half of a number of cases of influenza occurring during the epidemic of 1936–1937 and 1938–1939. The different strains isolated were much alike in cultural characteristics and in virulence. Nearly all produced the alpha type of hemolysis on horse blood agar plates, a few the beta type of hemolysis. When 0.1 cc. of a 1:200 or 1:1000 dilution of the primary dextrose brain broth culture of the streptococcus and of rapidly made subcultures in this medium were injected intracerebrally into rabbits and 0.03 cc. intracerebrally into mice, severe lesions of the mucous membrane of the trachea, bronchi, and the lungs developed. The lungs became greatly

distended and revealed widespread interspersed areas of emphysema, hemorrhagic edema and bronchopneumonia. Like injections of the streptococci from patients with diseases other than those of the respiratory tract seldom caused lesions of the lungs. An antiserum was prepared in horses by repeated injections of the streptococcus isolated in previous outbreaks and whose specificity was maintained throughout in a dense suspension of glycerol (2 parts) and 25 per cent salt solution (1 part). Nearly all patients suffering from influenza have yielded an immediate erythematous-edematous reaction on intradermal injection of approximately 0.03 cc. of a 10 per cent solution of the euglobulin fraction of this serum and no flares or slighter flares to like fractions of other antistreptococcal serums, antipneumococcus serums (types I and II) and normal horse serum, injected as controls. Positive ring precipitation tests with the serum and cleared nasopharyngeal washings from patients with influenza and the influenza antistreptococcal serum were obtained in nearly all cases. Control antistreptococcal serums yielded no clouding or lesser reactions. In a number of cases of typical influenza which did not develop pneumonia the streptococcus was isolated, and particularly marked skin reactions and positive precipitation reactions with the influenza antistreptococcal serums were obtained during the initial chill and lesser reactions in many other patients during the early stages of the disease, or in what is now considered as the virus phase of influenza. These results have been obtained in each of four widely separated outbreaks. The possible relation between the streptococcus and the virus is under study.

TISSUE RESPONSES TO RED BLOOD CELLS. E. L. Sarason (by invitation), R. M. Thomas and M. C. Winternitz, New Haven, Conn.

Abstract. Injection of homologous blood or 1 per cent Kaolin into the peritoneal cavity of rabbits, gauged to produce equivalent monocytic reaction, shows both fat and iron particles within the monocytes after blood, but not after Kaolin. The participation of the red blood cells in the production of the lipid of the exudate as well as the iron correlates with discoveries in diseases of the vessel wall.

THE ELECTRON MICROSCOPE AND ITS USE IN LOCALIZING MAGNESIUM AND CALCIUM IN TISSUE SECTIONS. Gordon H. Scott, St. Louis, Mo.

Abstract. When tissues are fixed by rapid freezing and dehydrating in vacuo at $-60°$ C. there is but slight chance of shifts from the original location of the inorganic salts. Tissues prepared in this manner are infiltrated with paraffin without having been exposed to air, and sections cut at 10 μ. These sections are placed on the cathode of an electron microscope and heated slowly until the salts begin to emit electrons. The electron beam is then passed through a pair of focussing magnetic fields and the resulting image thrown on a fluorescent screen. The source of electrons is easily recognizable as the treatment does not destroy topographical relations. Thus it is possible to distinguish in the image on the fluorescent screen cells, parts of tissues, and so on. Appropriate treatment of the cathode makes it possible to determine specifically the location of magnesium and of calcium. Skeletal muscle when prepared in this manner shows a distinct pattern of magnesium and calcium in the contraction bands of the fiber. Epithelium of the alimentary tract,

nerve cells and other tissues has also been examined and the magnesium and calcium localization ascertained. Further experiments designed to localize sodium and potassium are in progress.

MULTINUCLEATE GIANT CELLS WITH INCLUSION BODIES IN A FATAL CASE OF PRODROMAL MEASLES. K. H. Semsroth, Amsterdam, N. Y.

Abstract. A boy 20 months old was twice exposed to measles during 14 days. Two weeks after the first exposure acute dyspnea with respiratory death occurred. The clinical diagnosis was foreign body of the larynx, which was not corroborated at autopsy. The thymus, tonsils, hilum nodes and lymph follicles of the spleen showed numerous multinucleate giant cells of the type found to be pathognomic for measles by Warthin and others. In the epithelium of the respiratory passages degenerative changes were associated with proliferative changes. The latter consisted of the appearance of amitotic multinucleate epithelial giant cells comprising cytoplasmic inclusion bodies. Mononuclear peribronchiolitis and interlobular pulmonary edema were also present.